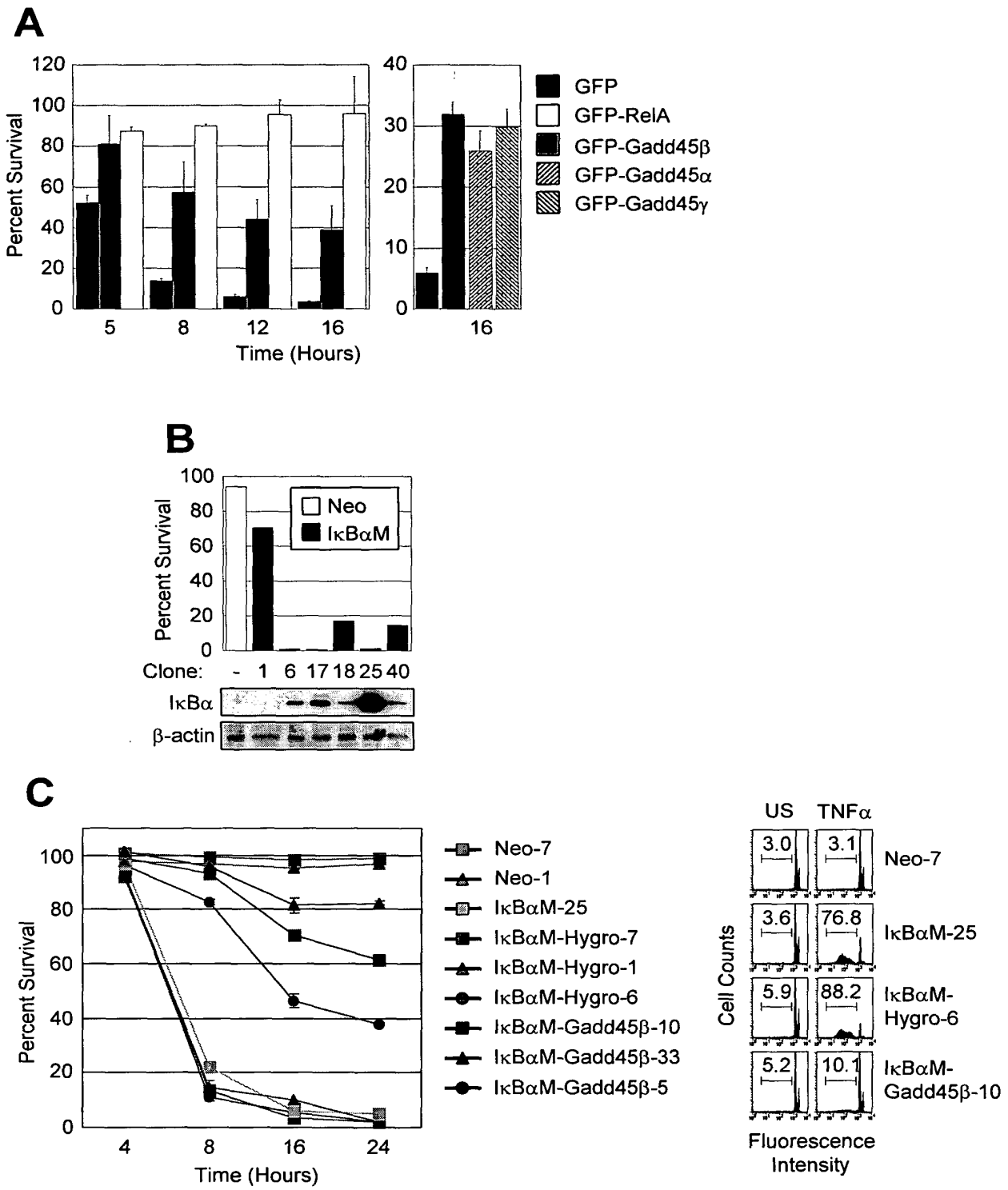
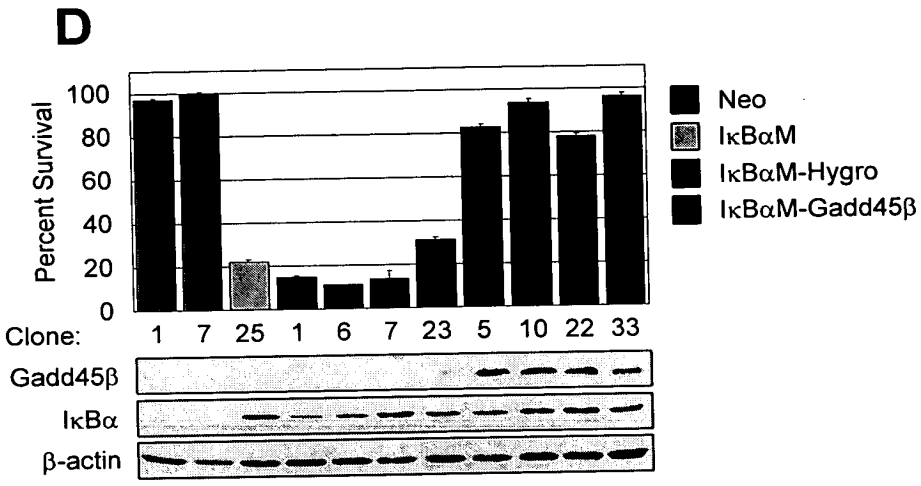
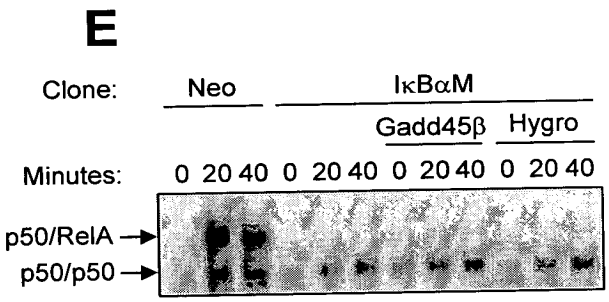

A
Percent Survival
120
100
80
60
40
20
0
5
8
12
16
Time (Hours)
40
30
20
10
0
16
GFP
GFP-RelA
GFP-Gadd45β
GFP-Gadd45α
GFP-Gadd45γ
B
Percent Survival
100
80
60
40
20
0
Neo
IκBαM
Clone: - 1 6 17 18 25 40
IκBα
β-actin
C
Percent Survival
100
80
60
40
20
0
4
8
16
24
Time (Hours)
Neo-7
Neo-1
IκBαM-25
IκBαM-Hygro-7
IκBαM-Hygro-1
IκBαM-Hygro-6
IκBαM-Gadd45β-10
IκBαM-Gadd45β-33
IκBαM-Gadd45β-5
US
TNFα
3.0
3.1
Neo-7
3.6
76.8
IκBαM-25
5.9
88.2
IκBαM-Hygro-6
5.2
10.1
IκBαM-Gadd45β-10
Cell Counts
Fluorescence Intensity

FIG. 1


D
Percent Survival
100
80
60
40
20
0
Clone: 1 7 25 1 6 7 23 5 10 22 33
Gadd45β
IκBα
β-actin
Neo
IκBαM
IκBαM-Hygro
IκBαM-Gadd45β


E
Clone: Neo IκBαM
Gadd45β Hygro
Minutes: 0 20 40 0 20 40 0 20 40 0 20 40
p50/RelA
p50/p50

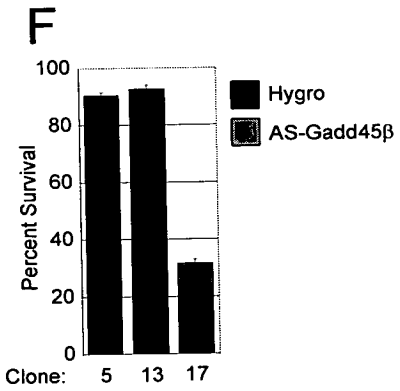

F
Percent Survival
100
80
60
40
20
0
Clone: 5 13 17
Hygro
AS-Gadd45β

FIG.1 Cont.

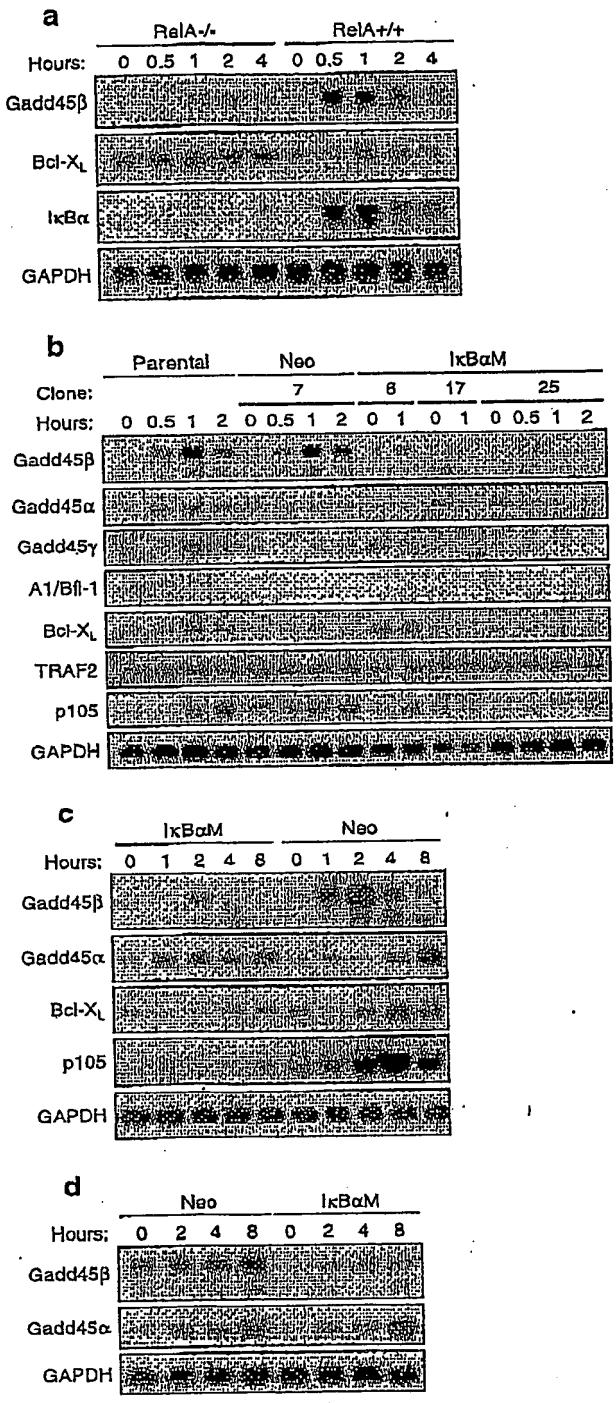

a
RelA-/-
RelA+/+
Hours: 0 0.5 1 2 4 0 0.5 1 2 4
Gadd45β
Bcl-XL
IκBα
GAPDH
b
Parental
Neo
IκBαM
Clone: 7 8 17 25
Hours: 0 0.5 1 2 0 0.5 1 2 0 1 0 1 0 0.5 1 2
Gadd45β
Gadd45α
Gadd45γ
A1/Bfl-1
Bcl-XL
TRAF2
p105
GAPDH
c
IκBαM
Neo
Hours: 0 1 2 4 8 0 1 2 4 8
Gadd45β
Gadd45α
Bcl-XL
p105
GAPDH
d
Neo
IκBαM
Hours: 0 2 4 8 0 2 4 8
Gadd45β
Gadd45α
GAPDH

FIG. 2

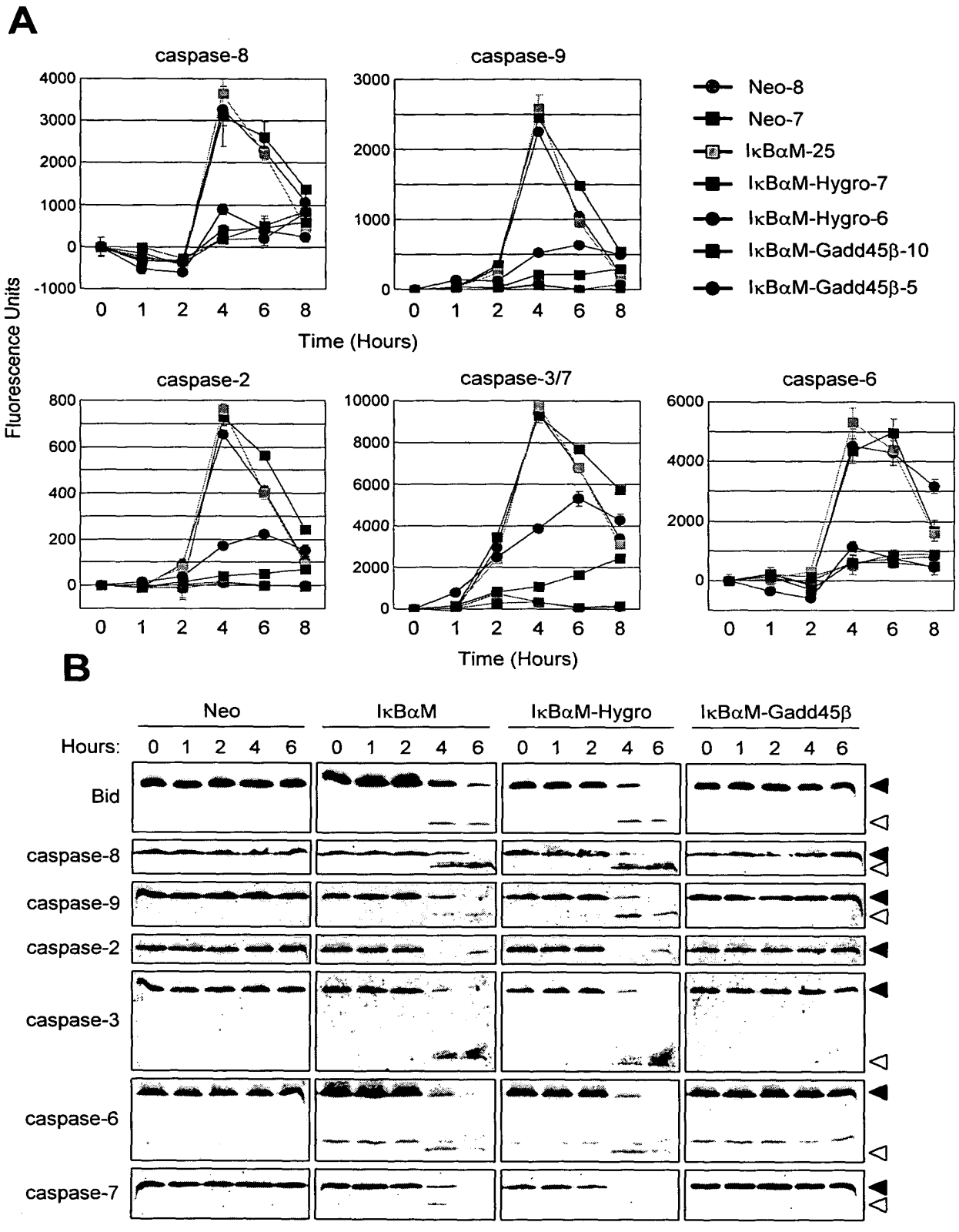
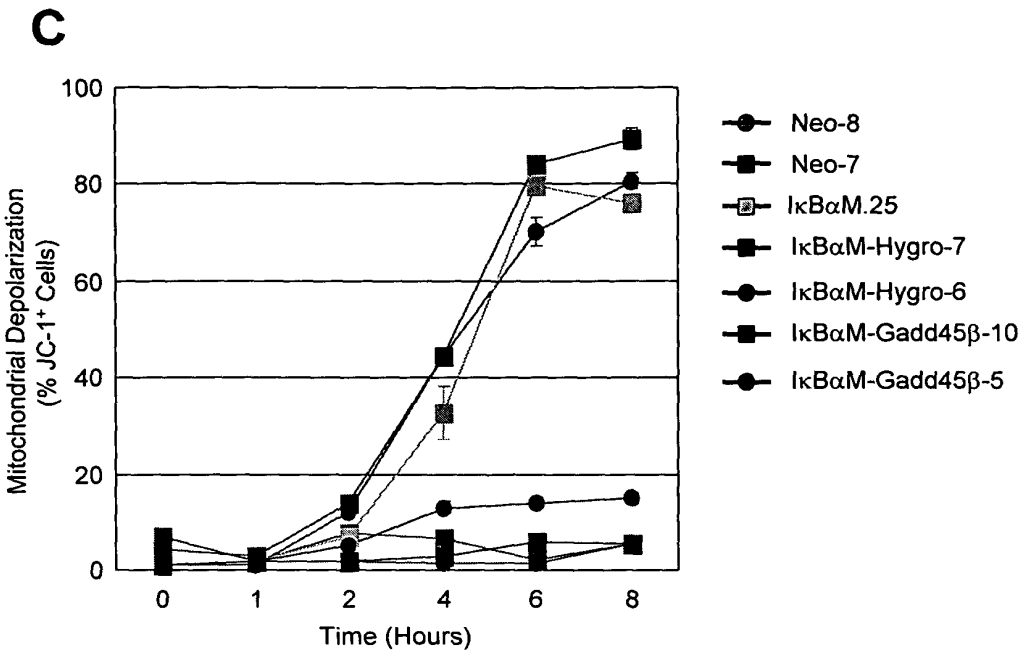
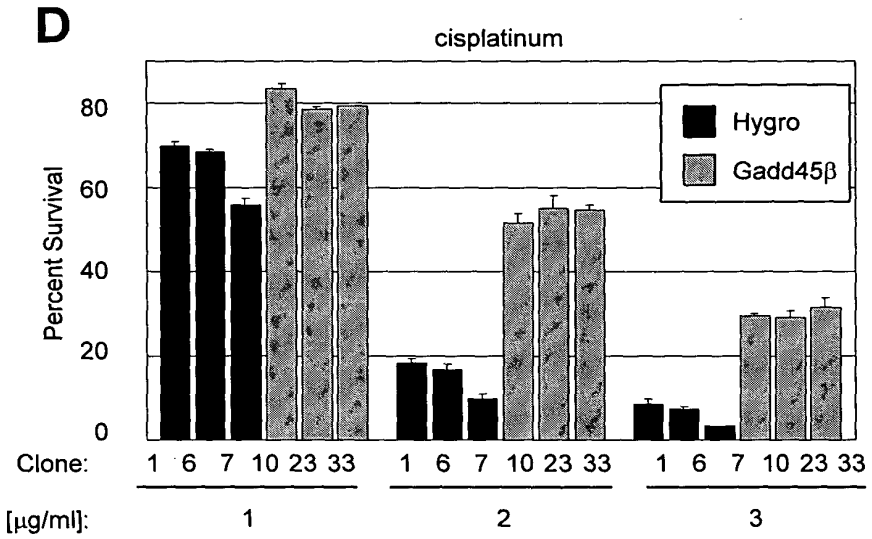

A
caspase-8
caspase-9
Neo-8
Neo-7
IκBαM-25
IκBαM-Hygro-7
IκBαM-Hygro-6
IκBαM-Gadd45β-10
IκBαM-Gadd45β-5
Fluorescence Units
Time (Hours)
caspase-2
caspase-3/7
caspase-6
Time (Hours)
B
Neo
IκBαM
IκBαM-Hygro
IκBαM-Gadd45β
Hours: 0 1 2 4 6
Bid
caspase-8
caspase-9
caspase-2
caspase-3
caspase-6
caspase-7

FIG. 3


C
Mitochondrial Depolarization (% JC-1+ Cells)
100
80
60
40
20
0
0
1
2
4
6
8
Time (Hours)
Neo-8
Neo-7
IκBαM.25
IκBαM-Hygro-7
IκBαM-Hygro-6
IκBαM-Gadd45β-10
IκBαM-Gadd45β-5


D
cisplatinum
Percent Survival
80
60
40
20
0
Hygro
Gadd45β
Clone:
1 6 7 10 23 33 1 6 7 10 23 33 1 6 7 10 23 33
[μg/ml]:
1
2
3

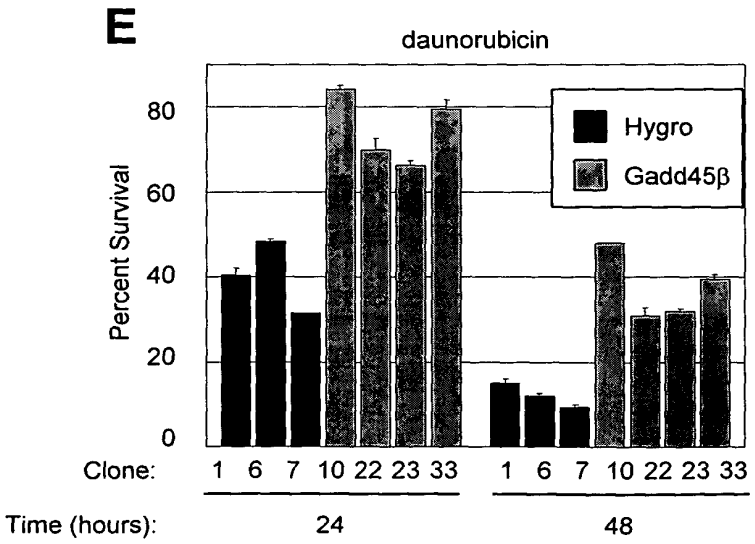

E
daunorubicin
Percent Survival
80
60
40
20
0
Hygro
Gadd45β
Clone:
1 6 7 10 22 23 33 1 6 7 10 22 23 33
Time (hours):
24
48

FIG. 3 cont.

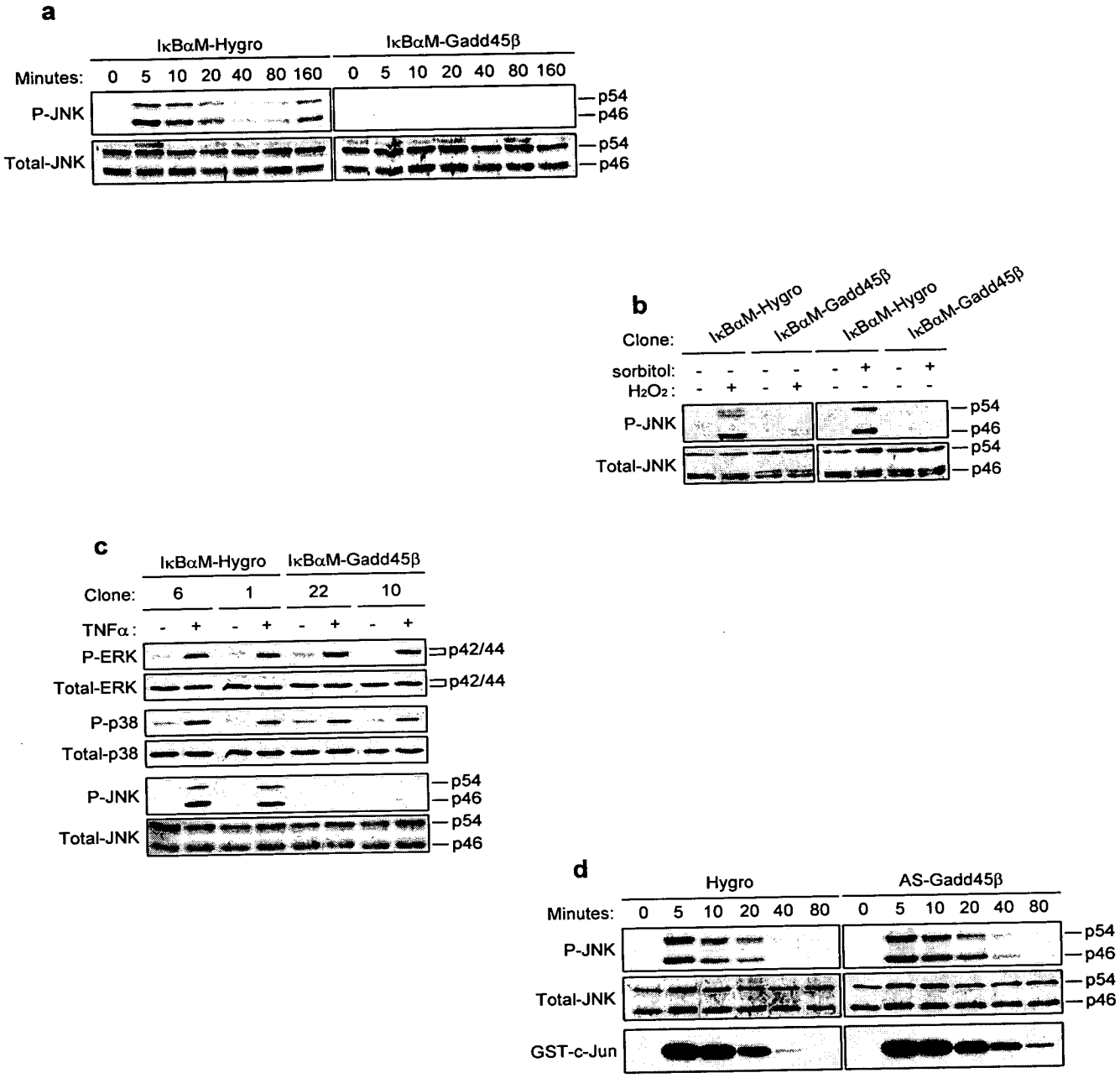
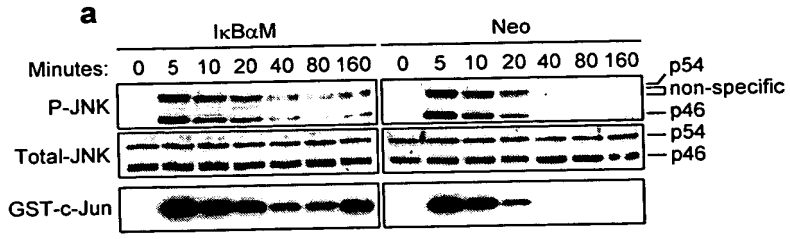
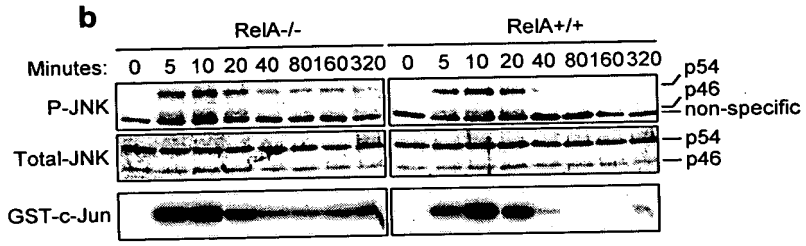
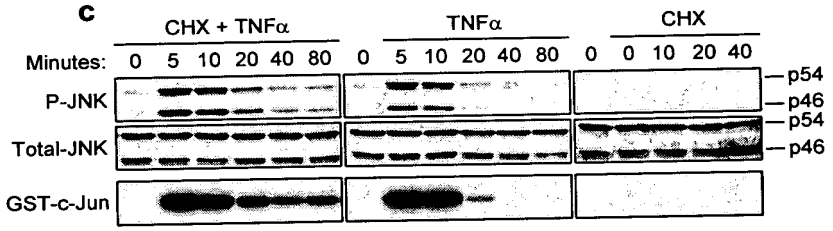
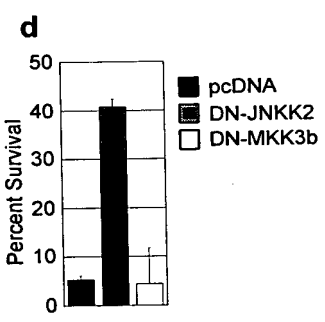

a
IκBαM-Hygro
IκBαM-Gadd45β
Minutes: 0 5 10 20 40 80 160 0 5 10 20 40 80 160
P-JNK
Total-JNK
p54
p46
b
Clone:
IκBαM-Hygro
IκBαM-Gadd45β
IκBαM-Hygro
IκBαM-Gadd45β
sorbitol:
H2O2:
P-JNK
Total-JNK
p54
p46
c
IκBαM-Hygro
IκBαM-Gadd45β
Clone: 6 1 22 10
TNFα:
P-ERK
Total-ERK
p42/44
P-p38
Total-p38
P-JNK
Total-JNK
p54
p46
d
Hygro
AS-Gadd45β
Minutes: 0 5 10 20 40 80 0 5 10 20 40 80
P-JNK
Total-JNK
GST-c-Jun
p54
p46

FIG. 4


a
IκBαM
Neo
Minutes: 0 5 10 20 40 80 160 0 5 10 20 40 80 160
P-JNK
Total-JNK
GST-c-Jun
p54
non-specific
p46
p54
p46


b
RelA-/-
RelA+/+
Minutes: 0 5 10 20 40 80160320 0 5 10 20 40 80160320
P-JNK
Total-JNK
GST-c-Jun
p54
p46
non-specific
p54
p46


c
CHX + TNFα
TNFα
CHX
Minutes: 0 5 10 20 40 80 0 5 10 20 40 80 0 0 10 20 40
P-JNK
Total-JNK
GST-c-Jun
p54
p46
p54
p46


d
Percent Survival
50
40
30
20
10
0
pcDNA
DN-JNKK2
DN-MKK3b

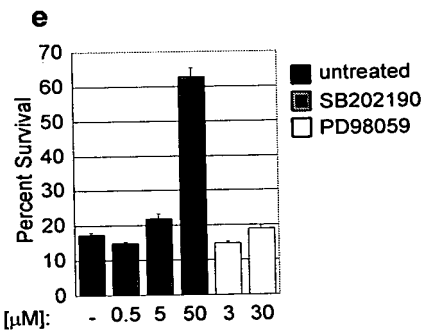

e
Percent Survival
70
60
50
40
30
20
10
0
[μM]: - 0.5 5 50 3 30
untreated
SB202190
PD98059

FIG. 5

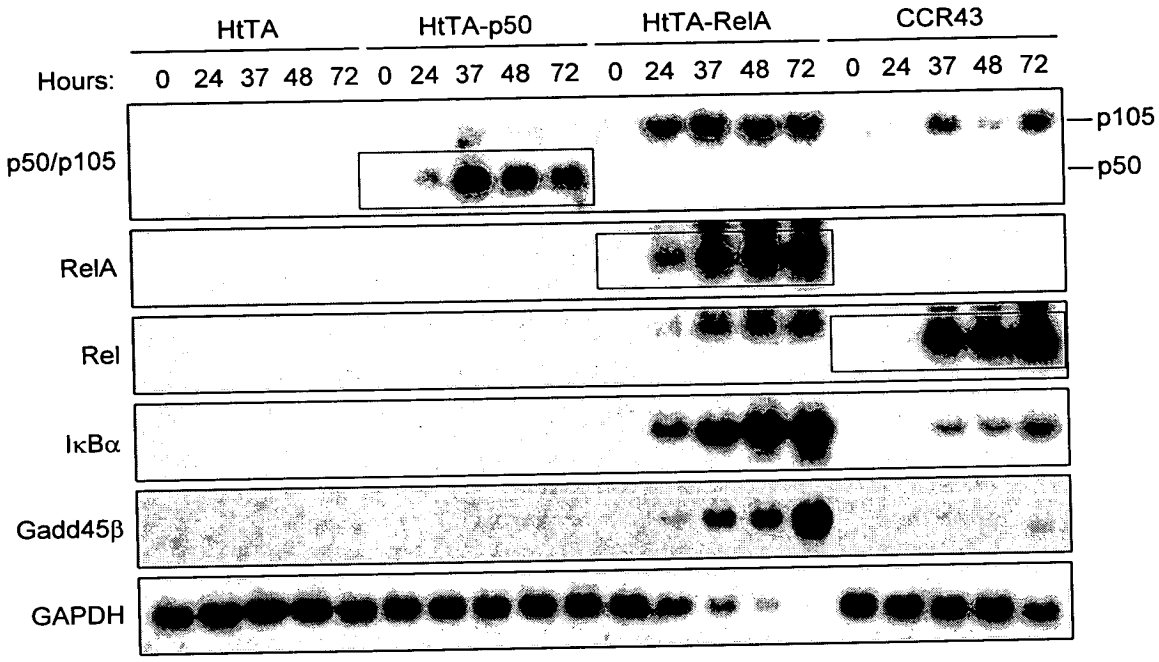

HtTA
HtTA-p50
HtTA-RelA
CCR43
Hours:
0 24 37 48 72 0 24 37 48 72 0 24 37 48 72 0 24 37 48 72
p50/p105
p105
p50
RelA
Rel
IκBα
Gadd45β
GAPDH

FIG. 6

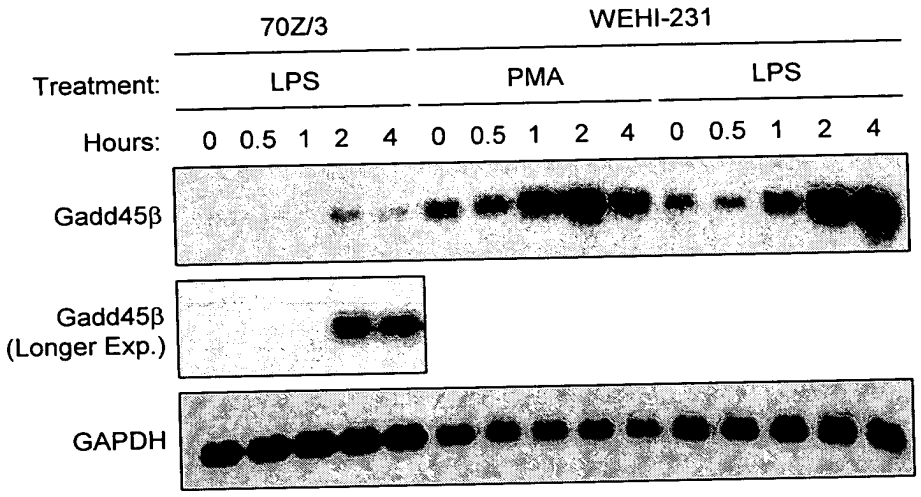

70Z/3
WEHI-231
Treatment:
LPS
PMA
LPS
Hours:
0 0.5 1 2 4 0 0.5 1 2 4 0 0.5 1 2 4
Gadd45β
Gadd45β
(Longer Exp.)
GAPDH

FIG. 7

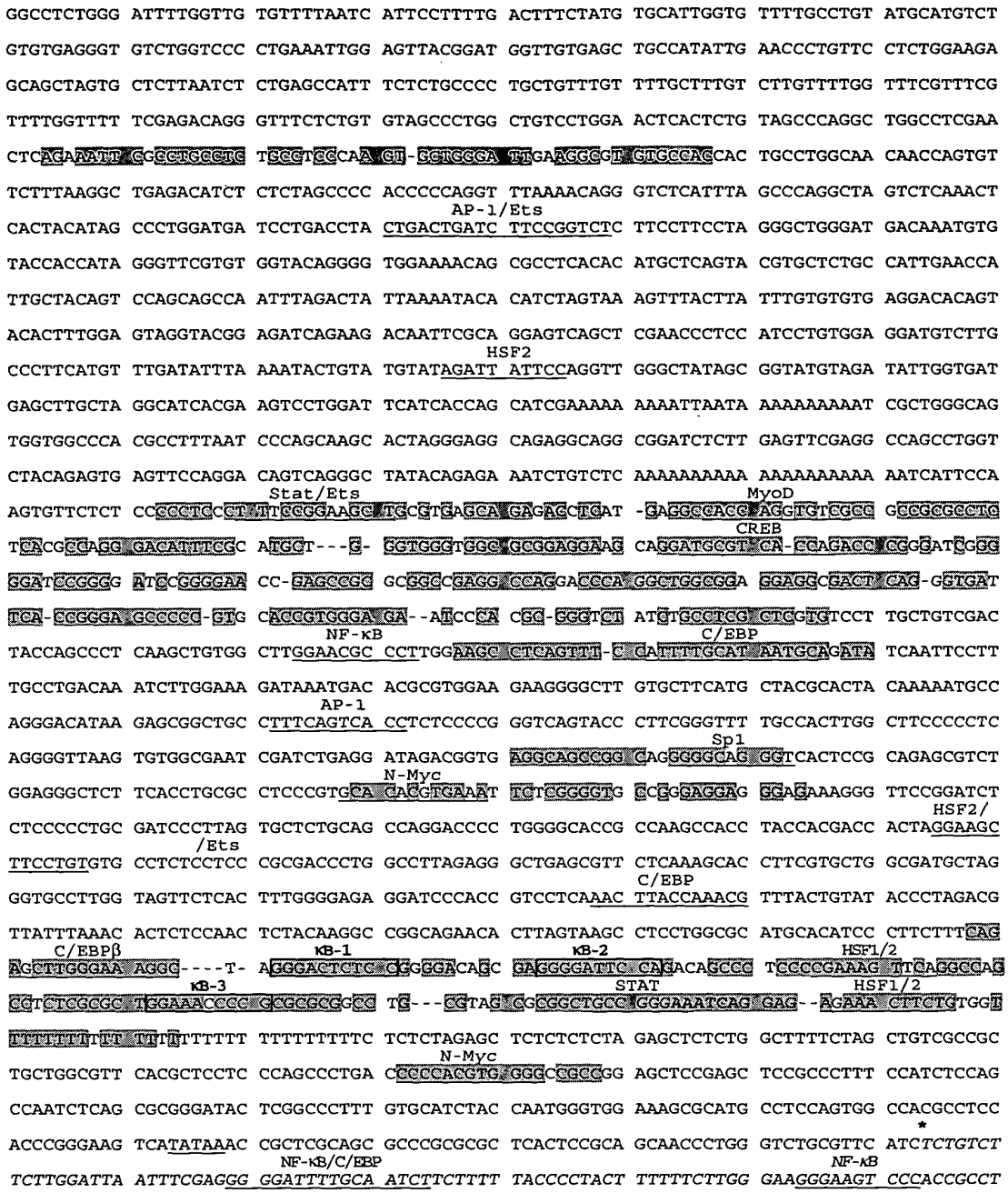

-2608 GGCCTCTGGG ATTTTGGTTG TGTTTTAATC ATTCCTTTTG ACTTTCTATG TGCATTGGTG TTTTGCCTGT ATGCATGTCT
-2528 GTGTGAGGGT GTCTGGTCCC CTGAAATTGG AGTTACGGAT GGTTGTGAGC TGCCATATTG AACCCTGTTC CTCTGGAAGA
-2448 GCAGCTAGTG CTCTTAATCT CTGAGCCATT TCTCTGCCCC TGCTGTTTGT TTTGCTTTGT CTTGTTTTGG TTTCGTTTCG
-2368 TTTTGGTTTT TCGAGACAGG GTTTCTCTGT GTAGCCCTGG CTGTCCTGGA ACTCACTCTG TAGCCCAGGC TGGCCTCGAA
-2288 CTCAGAAATT GCGCTGCCTG TGCGTCCCAA GT-GGTGCGA TTGAAGGCGT GTGCCAGCAC TGCCTGGCAA CAACCAGTGT
-2209 TCTTTAAGGC TGAGACATCT CTCTAGCCCC ACCCCCAGGT TTAAAACAGG GTCTCATTTA GCCCAGGCTA GTCTCAAACT
AP-1/Ets
-2129 CACTACATAG CCCTGGATGA TCCTGACCTA CTGACTGATC TTCCGGTCTC TTCCTTCCTA GGGCTGGGAT GACAAATGTG
-2049 TACCACCATA GGGTTCGTGT GGTACAGGGG TGGAAAACAG CGCCTCACAC ATGCTCAGTA CGTGCTCTGC CATTGAACCA
-1969 TTGCTACAGT CCAGCAGCCA ATTTAGACTA TTAAAATACA CATCTAGTAA AGTTTACTTA TTTGTGTGTG AGGACACAGT
-1889 ACACTTTGGA GTAGGTACGG AGATCAGAAG ACAATTCGCA GGAGTCAGCT CGAACCCTCC ATCCTGTGGA GGATGTCTTG
HSF2
-1809 CCCTTCATGT TTGATATTTA AAATACTGTA TGTATAGATT ATTCCAGGTT GGGCTATAGC GGTATGTAGA TATTGGTGAT
-1729 GAGCTTGCTA GGCATCACGA AGTCCTGGAT TCATCACCAG CATCGAAAAA AAAATTAATA AAAAAAAAAT CGCTGGGCAG
-1649 TGGTGGCCCA CGCCTTTAAT CCCAGCAAGC ACTAGGGAGG CAGAGGCAGG CGGATCTCTT GAGTTCGAGG CCAGCCTGGT
-1569 CTACAGAGTG AGTTCCAGGA CAGTCAGGGC TATACAGAGA AATCTGTCTC AAAAAAAAAA AAAAAAAAAA AATCATTCCA
Stat/Ets MyoD
-1489 AGTGTTCTCT CCGCCTCCCT TTCGGGAAGC TGCGTGAGCA GAGACTCAT -GAGGCGACG AGGTGTCGCG GCCGCGCCTG
CREB
-1410 TCACGCCAGG GACATTTCGC ATGGT---G- GGTGGGTGGC GCGGAGGAAG CAGGATGCGT CA-CCAGACC CGGGATCGGG
-1335 GGATCCGGGG ATCCGGGGAA CC-GAGCCGC GCGGCCGAGG CCAGGACCCA GGCTGGCGGA GGAGGCGACT CAG-GGTGAT
-1257 TCA-CCGGGA GCCCCC-GTG CACCGTGGGA GA--ATCCCA CGC-GGGTCT ATGTGCCTCG GTCGTGTCCT TGCTGTCGAC
NF-κB C/EBP
-1182 TACCAGCCCT CAAGCTGTGG CTTGGAACGC CCTTGGAAGC CTCAGTTT-C CATTTTGCAT AATGCAGATA TCAATTCCTT
-1103 TGCCTGACAA ATCTTGGAAA GATAAATGAC ACGCGTGGAA GAAGGGGCTT GTGCTTCATG CTACGCACTA CAAAAATGCC
AP-1
-1023 AGGGACATAA GAGCGGCTGC CTTTCAGTCA CCTCTCCCCG GGTCAGTACC CTTCGGGTTT TGCCACTTGG CTTCCCCCTC
Sp1
-943 AGGGGTTAAG TGTGGCGAAT CGATCTGAGG ATAGACGGTG AGGCAGCCGG CAGGGGGCAG GGTCACTCCG CAGAGCGTCT
N-Myc
-863 GGAGGGCTCT TCACCTGCGC CTCCCGTGCA CACGTGAAAT TGTCGGGGTG CCGGGAGGAG GGAGAAAGGG TTCCGGATCT
HSF2/
-783 CTCCCCCTGC GATCCCTTAG TGCTCTGCAG CCAGGACCCC TGGGGCACCG CCAAGCCACC TACCACGACC ACTAGGAAGC
/Ets
-703 TTCCTGTGTG CCTCTCCTCC CGCGACCCTG GCCTTAGAGG GCTGAGCGTT CTCAAAGCAC CTTCGTGCTG GCGATGCTAG
C/EBP
-623 GGTGCCTTGG TAGTTCTCAC TTTGGGGAGA GGATCCCACC GTCCTCAAAC TTACCAAACG TTTACTGTAT ACCCTAGACG
-543 TTATTTAAAC ACTCTCCAAC TCTACAAGGC CGGCAGAACA CTTAGTAAGC CTCCTGGCGC ATGCACATCC CTTCTTTCAG
C/EBPβ κB-1 κB-2 HSF1/2
-463 AGCTTGGGAA AGGG----T- AGGGACTCTC CGGGGACAGC GAGGGGATTC CAGACAGCCC TCCCCGAAAG TTCAGGCCAG
κB-3 STAT HSF1/2
-388 CGTCTCGCGC TGGAAACCCC GCGCGCGGCC TG---CGTAGT CGCGGCTGCC GGGAAATCAG GAG--AGAAA CTTCTGTGGT
-313 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTC TCTCTAGAGC TCTCTCTCTA GAGCTCTCTG GCTTTTCTAG CTGTCGCCGC
N-Myc
-233 TGCTGGCGTT CACGCTCCTC CCAGCCCTGA CCCCCACGTG GGGCCGCCGG AGCTCCGAGC TCCGCCCTTT CCATCTCCAG
-153 CCAATCTCAG CGCGGGATAC TCGGCCCTTT GTGCATCTAC CAATGGGTGG AAAGCGCATG CCTCCAGTGG CCACGCCTCC
-73 ACCCGGGAAG TCATATAAAC CGCTCGCAGC GCCCGCGCGC TCACTCCGCA GCAACCCTGG GTCTGCGTTC ATCTCTGTCT
NF-κB/C/EBP NF-κB
+8 TCTTGGATTA ATTTCGAGGG GGATTTTGCA ATCTTCTTTT TACCCCTACT TTTTTCTTGG GAAGGGAAGT CCCACCGCCT

FIG. 8

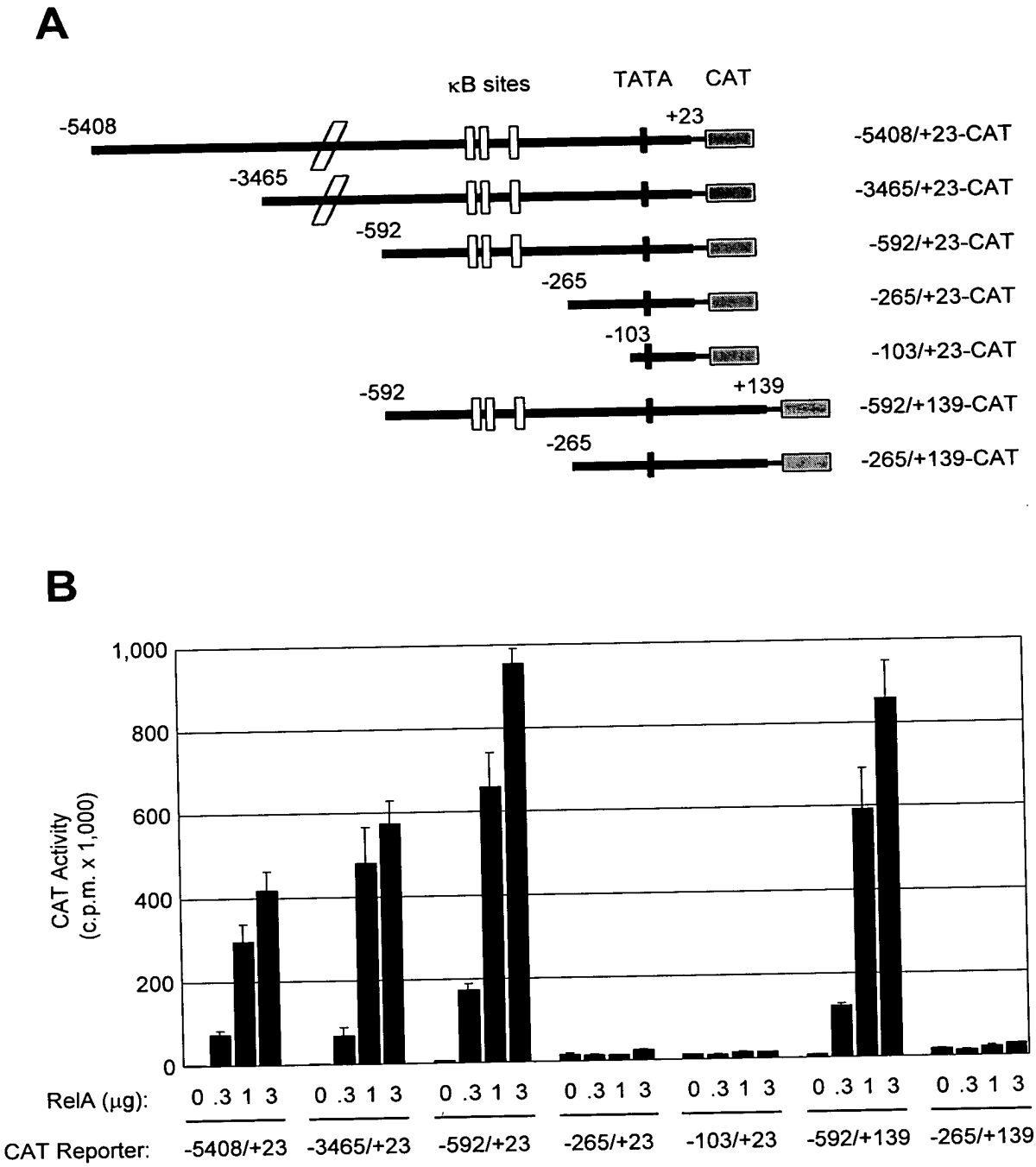
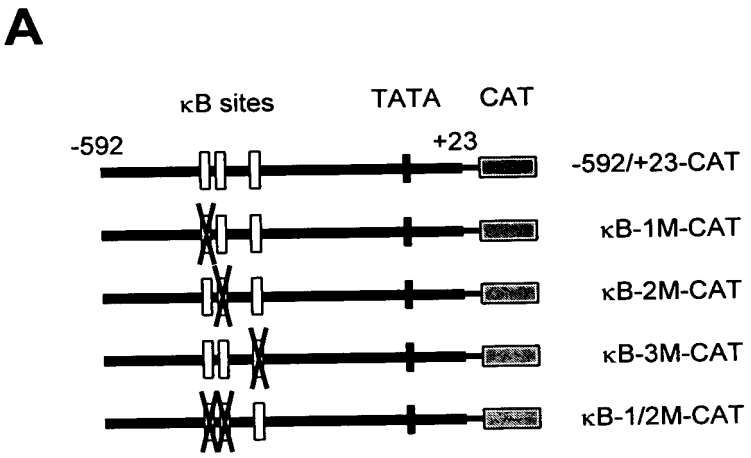

A
κB sites
TATA
CAT
-5408
+23
-5408/+23-CAT
-3465
-3465/+23-CAT
-592
-592/+23-CAT
-265
-265/+23-CAT
-103
-103/+23-CAT
-592
+139
-592/+139-CAT
-265
-265/+139-CAT
B
CAT Activity
(c.p.m. x 1,000)
1,000
800
600
400
200
0
RelA (μg):
0 .3 1 3
CAT Reporter:
-5408/+23
-3465/+23
-592/+23
-265/+23
-103/+23
-592/+139
-265/+139

FIG. 9


A
κB sites
TATA
CAT
-592
+23
-592/+23-CAT
κB-1M-CAT
κB-2M-CAT
κB-3M-CAT
κB-1/2M-CAT

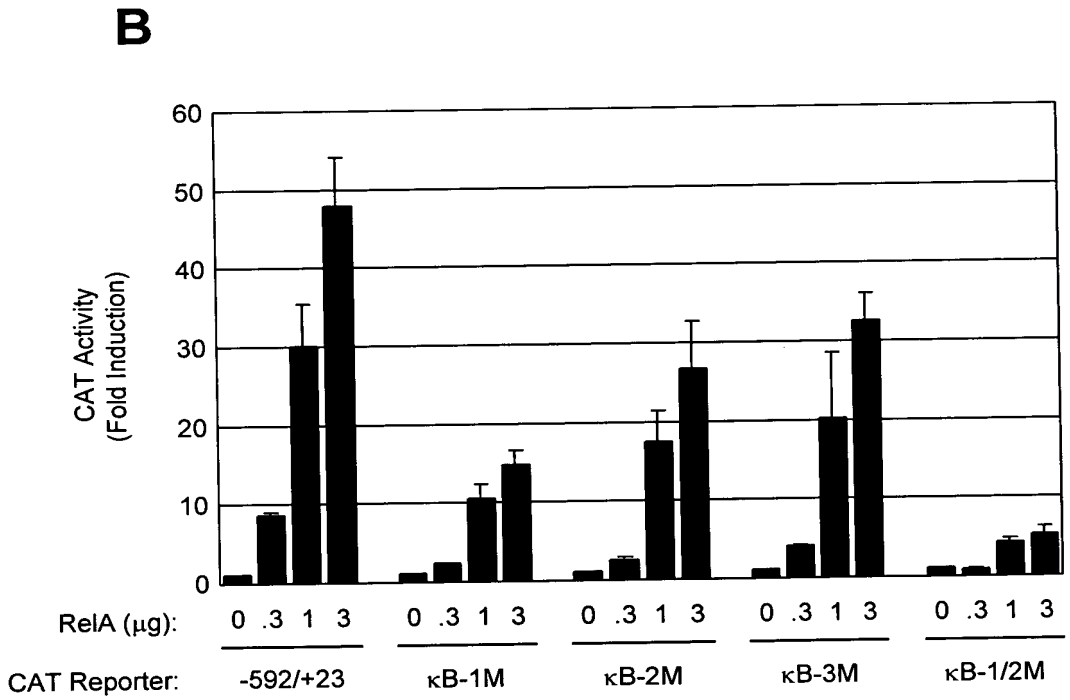

B
CAT Activity
(Fold Induction)
60
50
40
30
20
10
0
RelA (μg):
0 .3 1 3
0 .3 1 3
0 .3 1 3
0 .3 1 3
0 .3 1 3
CAT Reporter:
-592/+23
κB-1M
κB-2M
κB-3M
κB-1/2M

FIG. 10

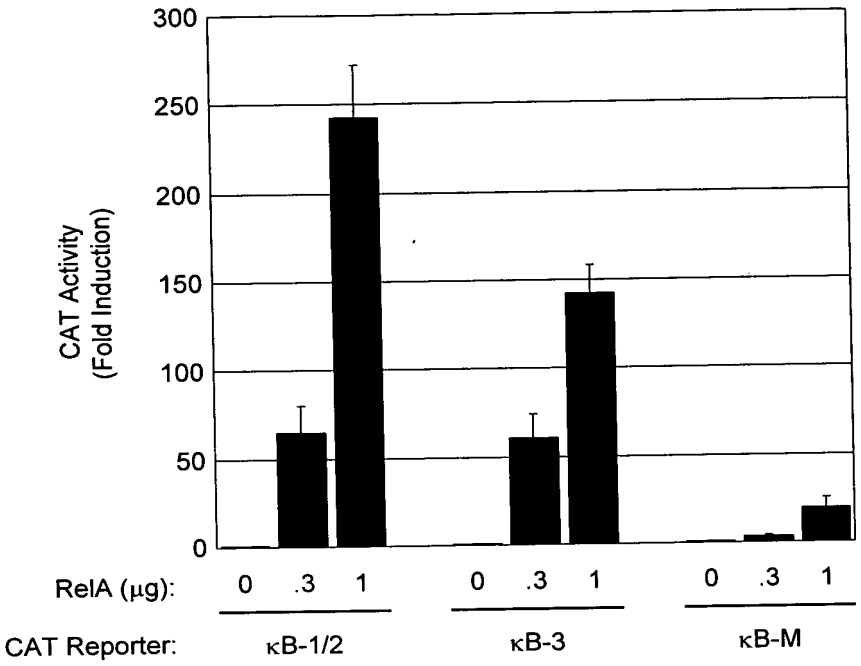

300
250
200
150
100
50
0
CAT Activity
(Fold Induction)
RelA (μg):
0 .3 1
0 .3 1
0 .3 1
CAT Reporter:
κB-1/2
κB-3
κB-M

FIG. 11

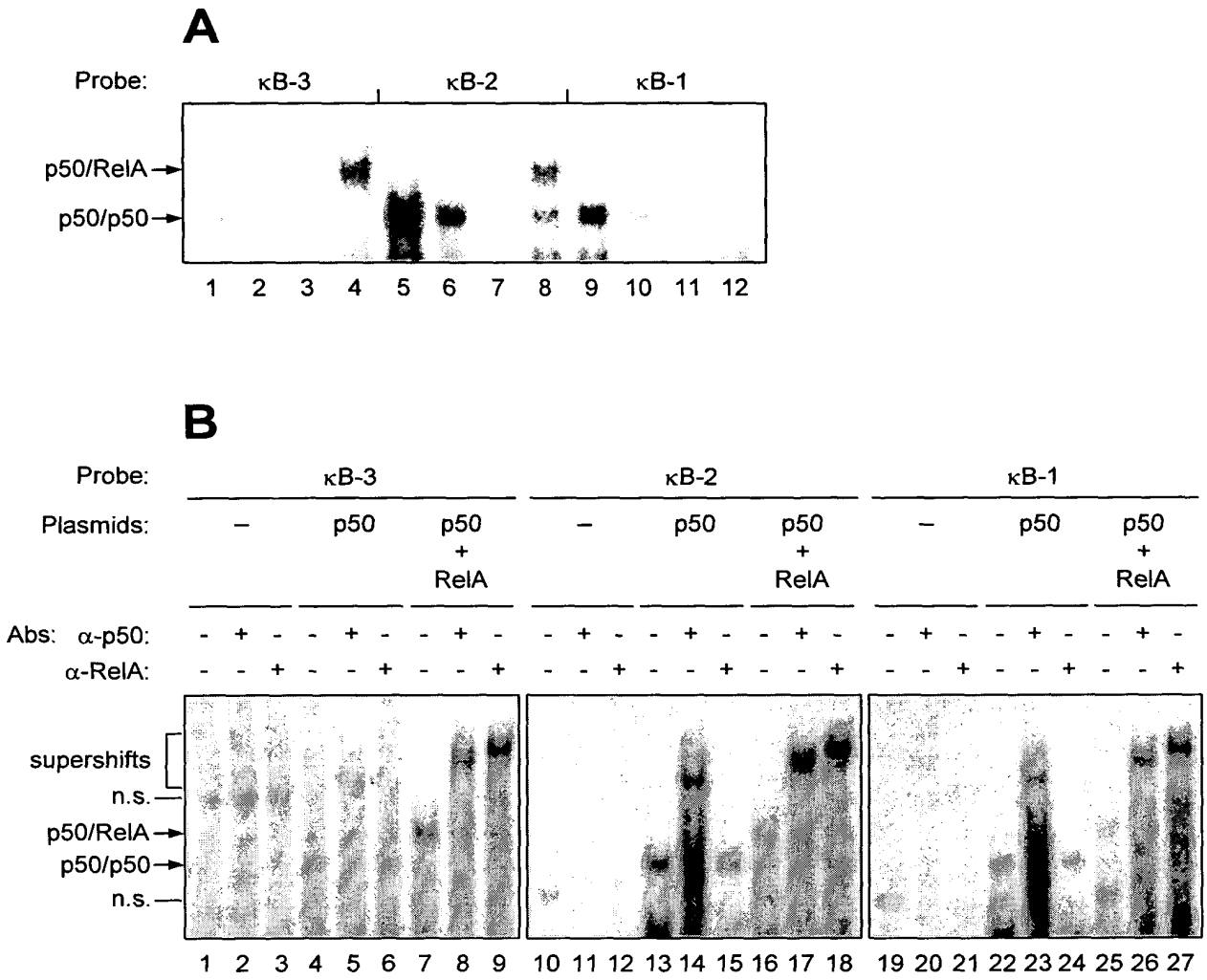

A
Probe:
κB-3
κB-2
κB-1
p50/RelA
p50/p50
1 2 3 4 5 6 7 8 9 10 11 12
B
Probe:
κB-3
κB-2
κB-1
Plasmids:
–
p50
p50 + RelA
Abs: α-p50:
α-RelA:
supershifts
n.s.
p50/RelA
p50/p50
n.s.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

FIG. 12

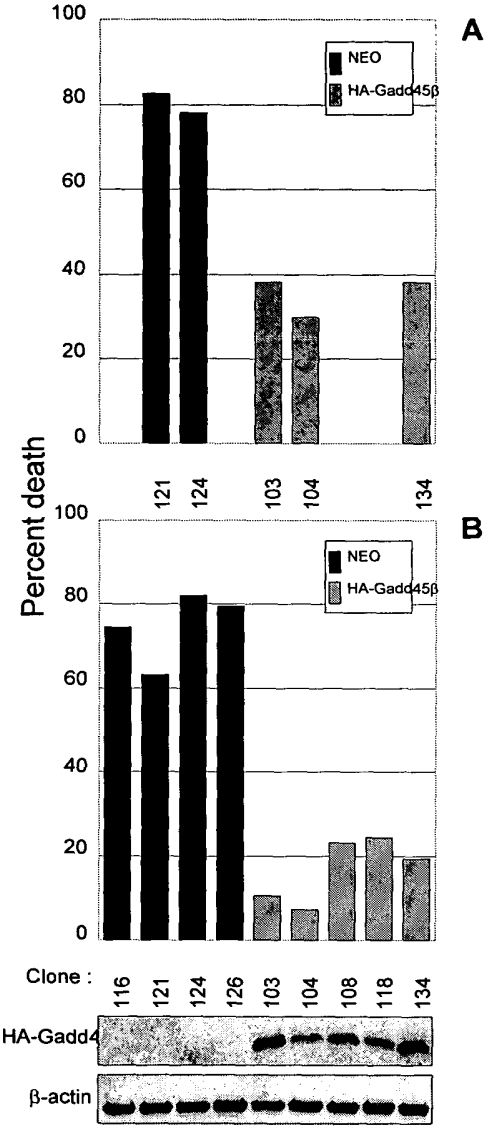

A
B
NEO
HA-Gadd45β
Percent death
100
80
60
40
20
0
121
124
103
104
134
Clone :
116
121
124
126
103
104
108
118
134
HA-Gadd4
β-actin

FIG. 13

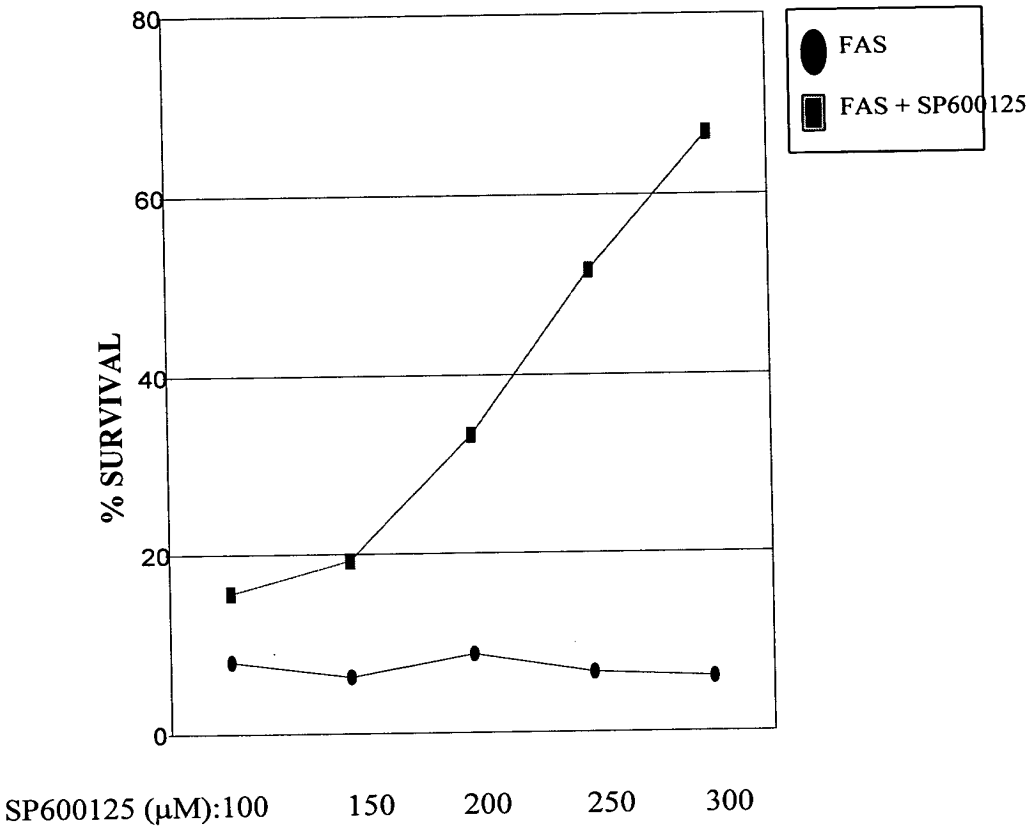

80
60
40
20
0
% SURVIVAL
FAS
FAS + SP600125
SP600125 (μM):100
150
200
250
300

FIG. 14

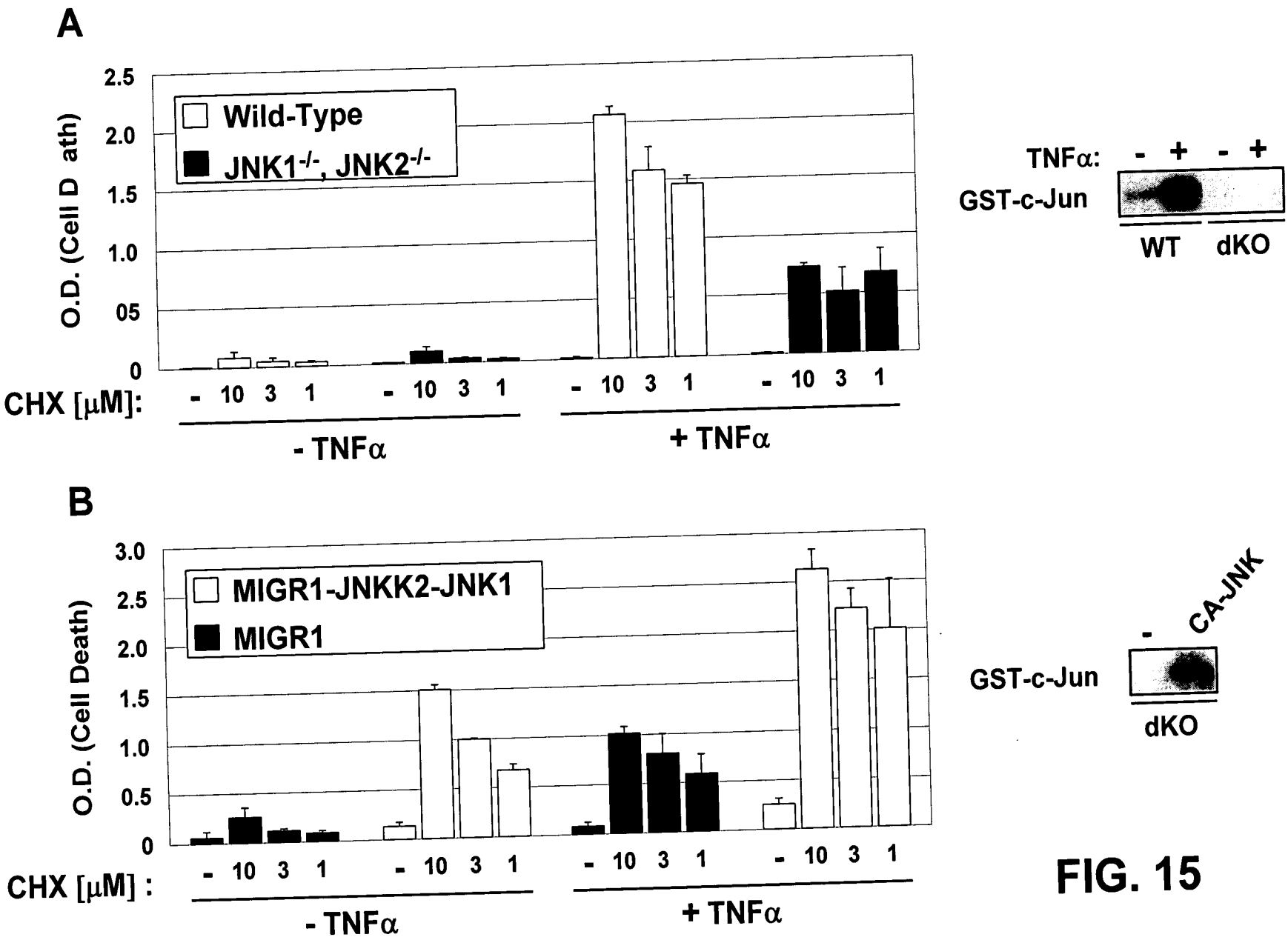

A
2.5
2.0
1.5
1.0
05
0
O.D. (Cell D ath)
Wild-Type
JNK1-/-, JNK2-/-
CHX [μM]:
- 10 3 1 - 10 3 1 - 10 3 1 - 10 3 1
- TNFα
+ TNFα
TNFα: - + - +
GST-c-Jun
WT dKO
B
3.0
2.5
2.0
1.5
1.0
0.5
0
O.D. (Cell Death)
MIGR1-JNKK2-JNK1
MIGR1
CHX [μM] :
- 10 3 1 - 10 3 1 - 10 3 1 - 10 3 1
- TNFα
+ TNFα
- CA-JNK
GST-c-Jun
dKO

FIG. 15

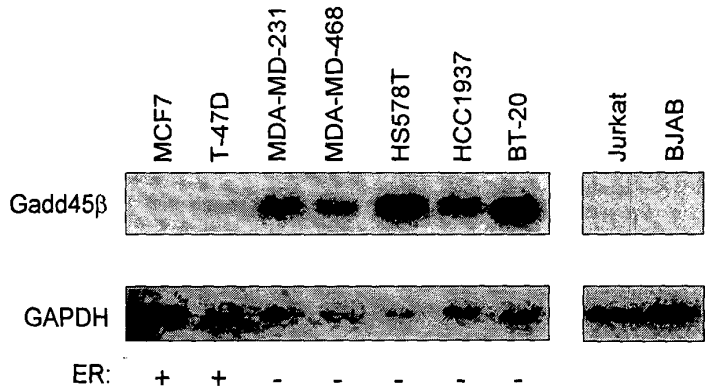

MCF7
T-47D
MDA-MD-231
MDA-MD-468
HS578T
HCC1937
BT-20
Jurkat
BJAB
Gadd45β
GAPDH
ER:
+ + - - - - -

FIG. 16

MDA-MD 231

	SP600125		
	0	100μM	50μM
CAPE (50 μg/ml)	-	+++	+++
Parthenolide (2.5 μg/ml)	-	+++	++++
Prostaglandin A_1 (100μM)	+	++++	++++

BT-20

	SP600125		
	0	100μM	50μM
CAPE (50 μg/ml)	+	N.D.	+++
Parthenolide (10 μg/ml)	-	+++	++++
Prostaglandin A_1 (100μM)	+	+++	+++

FIG. 17

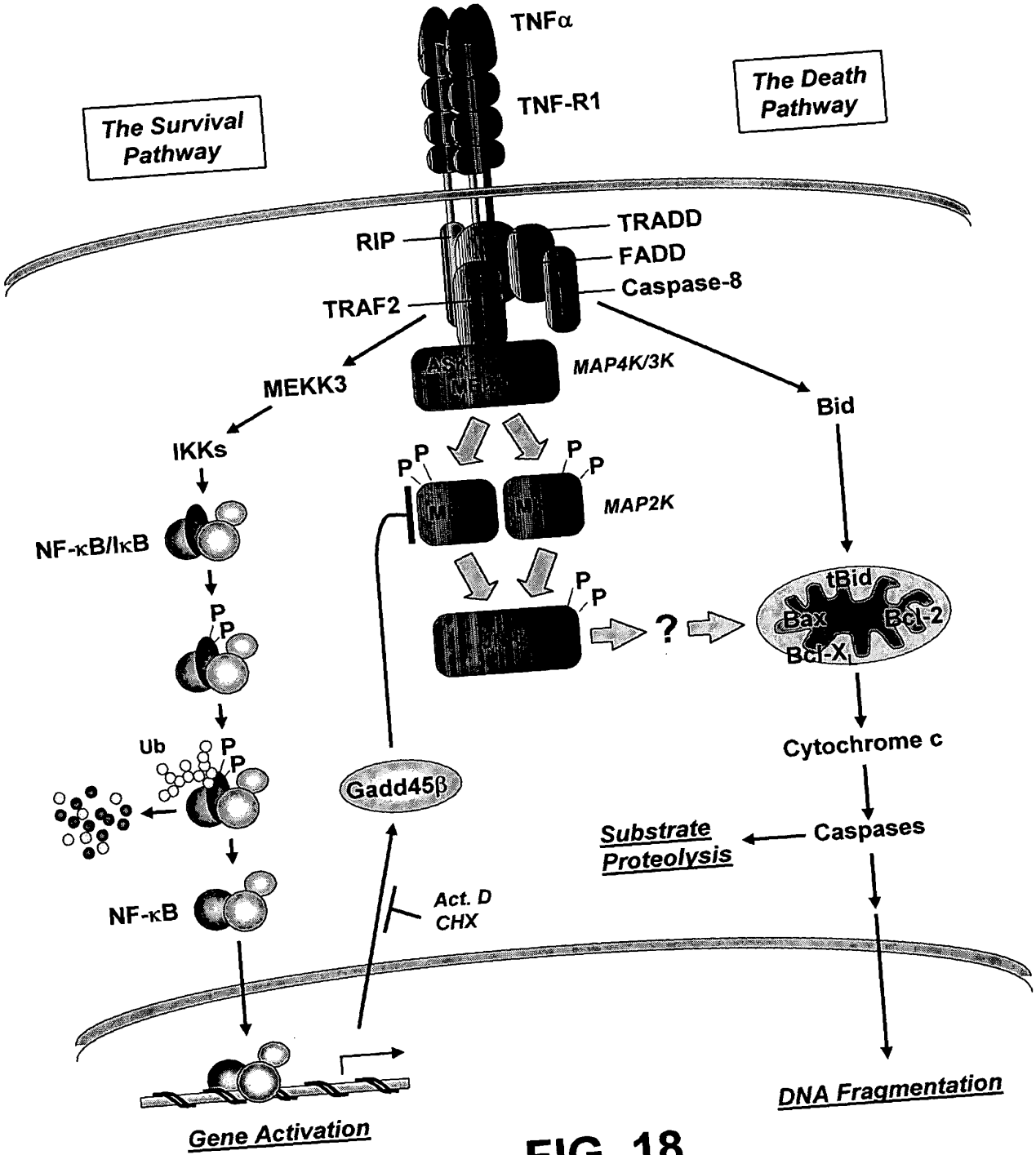

TNFα
TNF-R1
The Survival Pathway
The Death Pathway
TRADD
RIP
FADD
Caspase-8
TRAF2
MAP4K/3K
MEKK3
Bid
IKKs
MAP2K
NF-κB/IκB
tBid
Bax
Bcl-2
Bcl-X
Ub
Cytochrome c
Gadd45β
Substrate Proteolysis
Caspases
Act. D
CHX
NF-κB
Gene Activation
DNA Fragmentation

FIG. 18

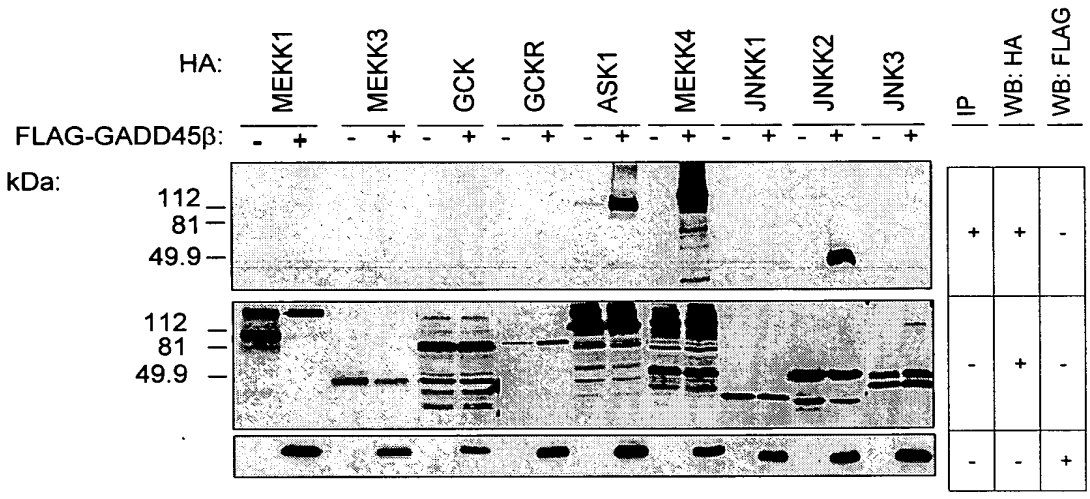

HA:
MEKK1
MEKK3
GCK
GCKR
ASK1
MEKK4
JNKK1
JNKK2
JNK3
IP
WB: HA
WB: FLAG
FLAG-GADD45β:
- + - + - + - + - + - + - + - + - +
kDa:
112
81
49.9
112
81
49.9
+ + -
- + -
- - +

FIG. 19

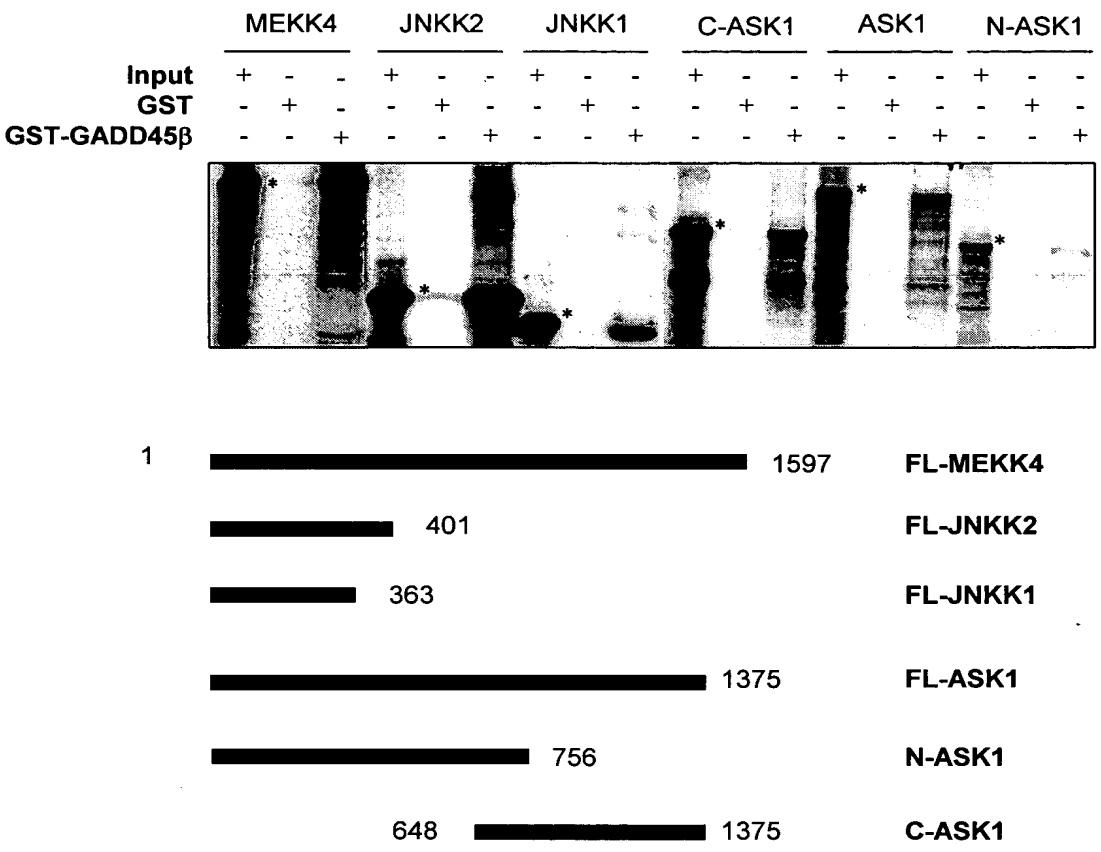

MEKK4
JNKK2
JNKK1
C-ASK1
ASK1
N-ASK1
Input
GST
GST-GADD45β
1
1597
FL-MEKK4
401
FL-JNKK2
363
FL-JNKK1
1375
FL-ASK1
756
N-ASK1
648
1375
C-ASK1

FIG. 20

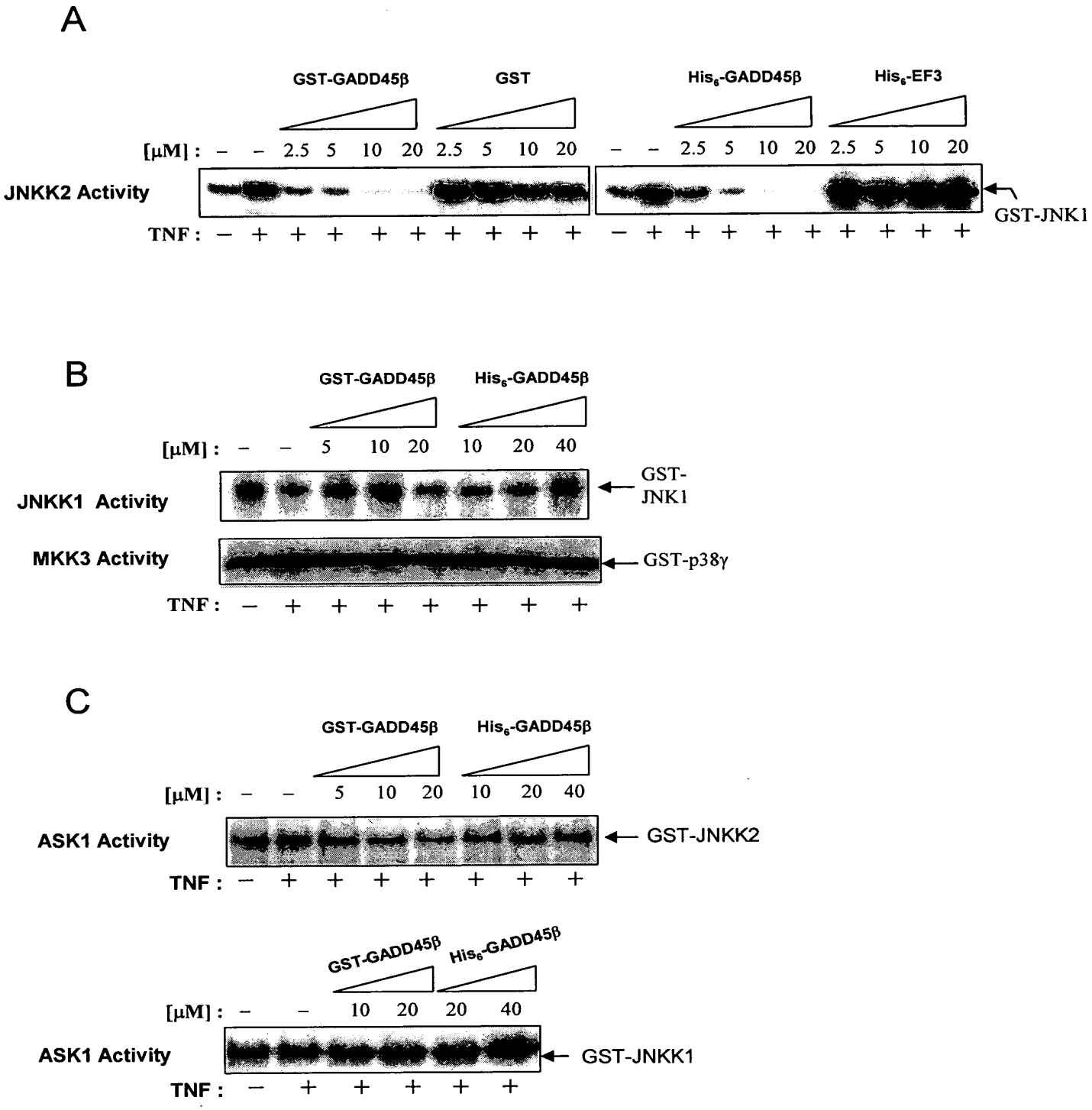

A
GST-GADD45β
GST
His6-GADD45β
His6-EF3
[μM] : – – 2.5 5 10 20 2.5 5 10 20 – – 2.5 5 10 20 2.5 5 10 20
JNKK2 Activity
GST-JNK1
TNF : – + + + + + + + + + – + + + + + + + + +
B
GST-GADD45β
His6-GADD45β
[μM] : – – 5 10 20 10 20 40
JNKK1 Activity
GST-JNK1
MKK3 Activity
GST-p38γ
TNF : – + + + + + + +
C
GST-GADD45β
His6-GADD45β
[μM] : – – 5 10 20 10 20 40
ASK1 Activity
GST-JNKK2
TNF : – + + + + + + +
GST-GADD45β
His6-GADD45β
[μM] : – – 10 20 20 40
ASK1 Activity
GST-JNKK1
TNF : – + + + + +

FIG. 21

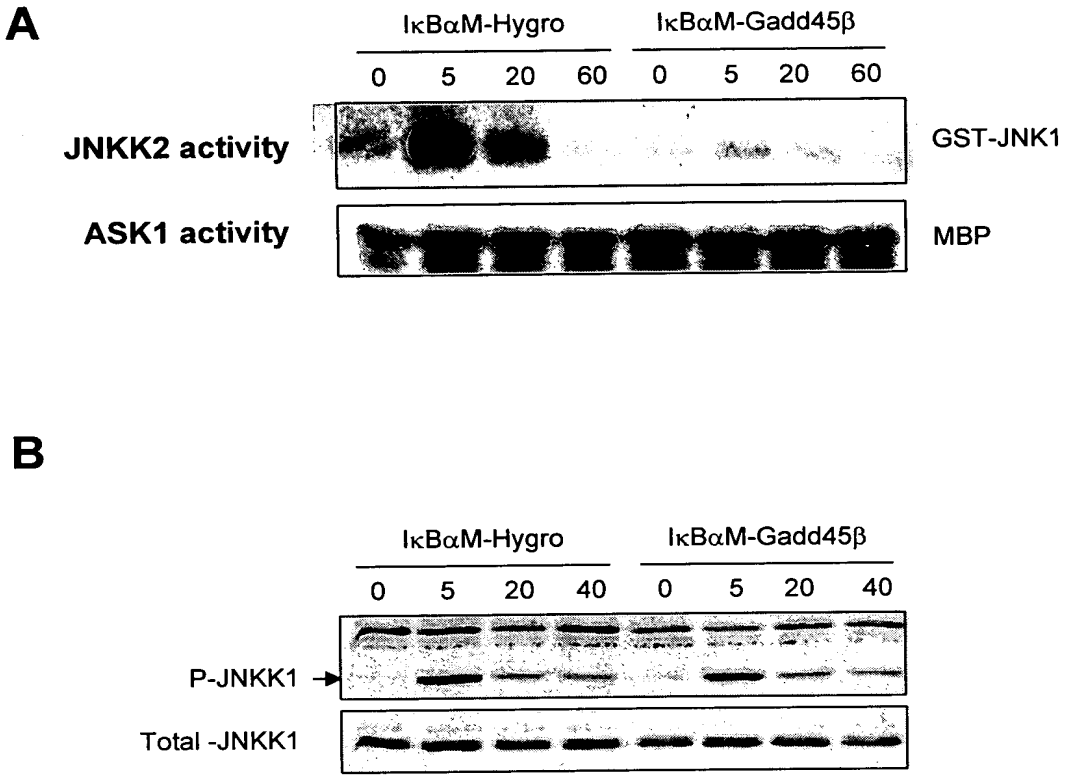

A
IκBαM-Hygro
IκBαM-Gadd45β
0 5 20 60 0 5 20 60
JNKK2 activity
GST-JNK1
ASK1 activity
MBP
B
IκBαM-Hygro
IκBαM-Gadd45β
0 5 20 40 0 5 20 40
P-JNKK1
Total -JNKK1

FIG. 22

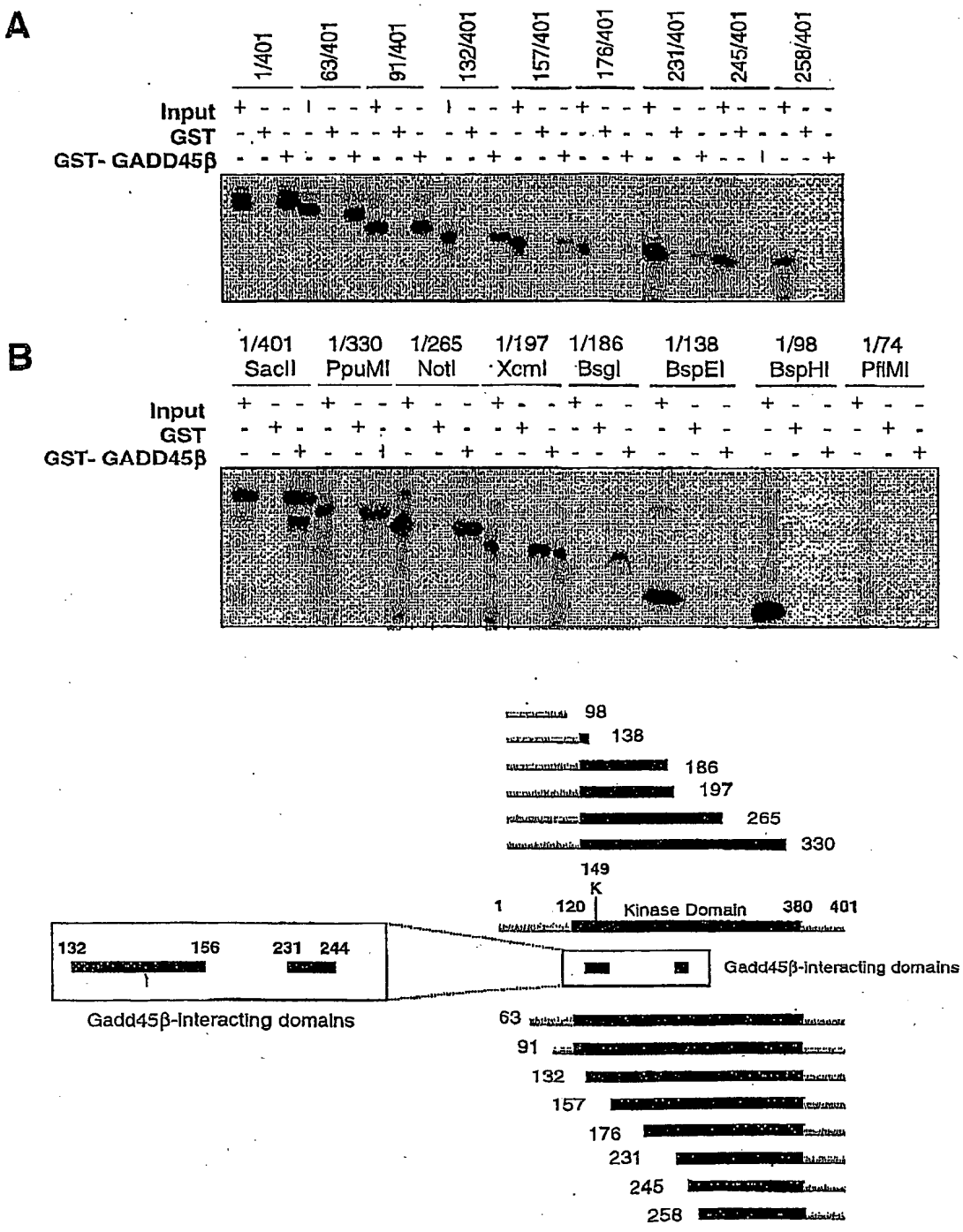

A
1/401
63/401
91/401
132/401
157/401
176/401
231/401
245/401
258/401
Input
GST
GST- GADD45β
B
1/401 SacII
1/330 PpuMI
1/265 NotI
1/197 XcmI
1/186 BsgI
1/138 BspEI
1/98 BspHI
1/74 PflMI
Input
GST
GST- GADD45β
98
138
186
197
265
330
149
K
1
120
Kinase Domain
380
401
132
156
231
244
Gadd45β-interacting domains
Gadd45β-interacting domains
63
91
132
157
176
231
245
258

FIG. 23

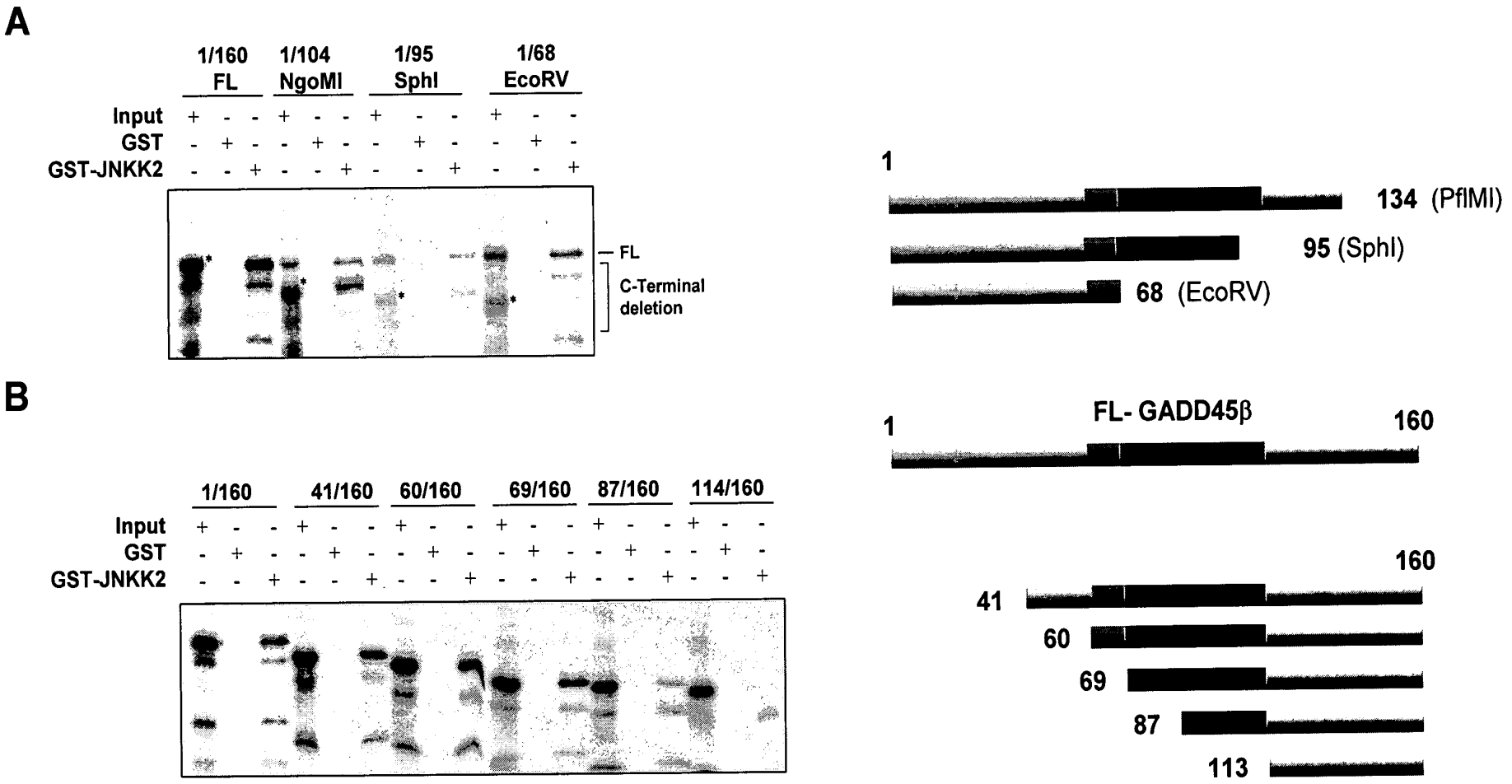
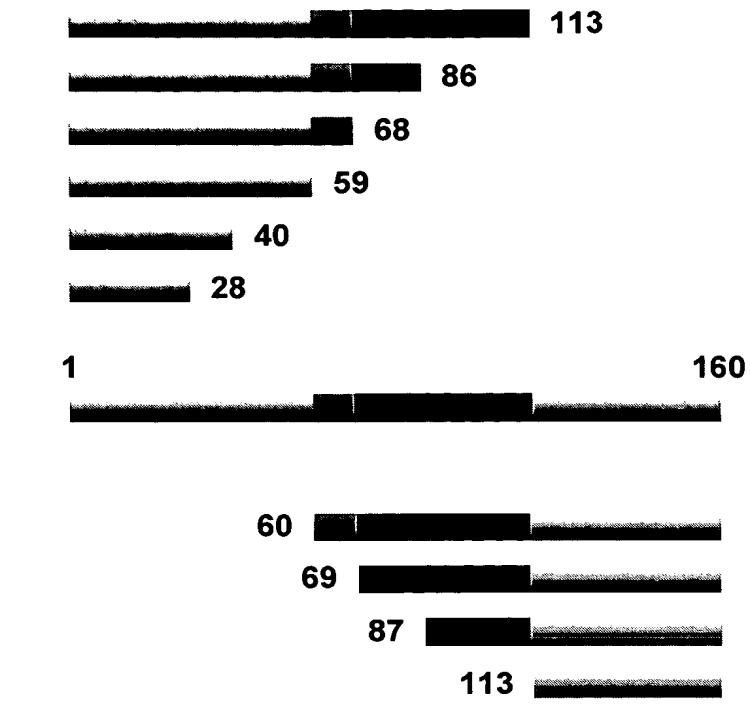
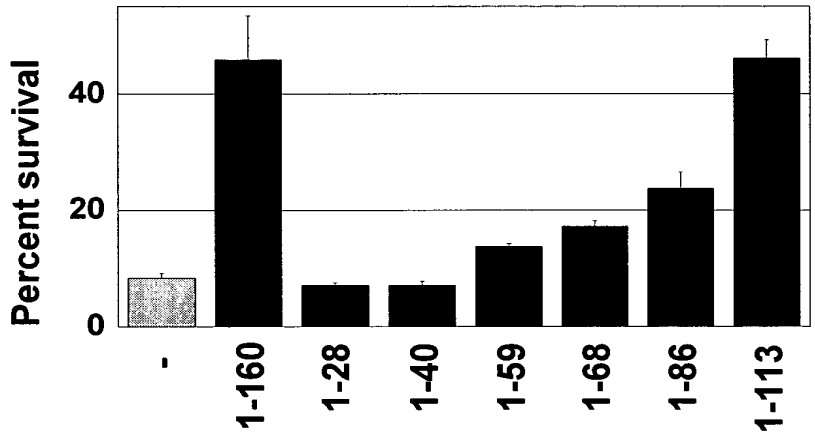


FIG. 24


113
86
68
59
40
28
1
160
60
69
87
113


Percent survival
40
20
0
-
1-160
1-28
1-40
1-59
1-68
1-86
1-113

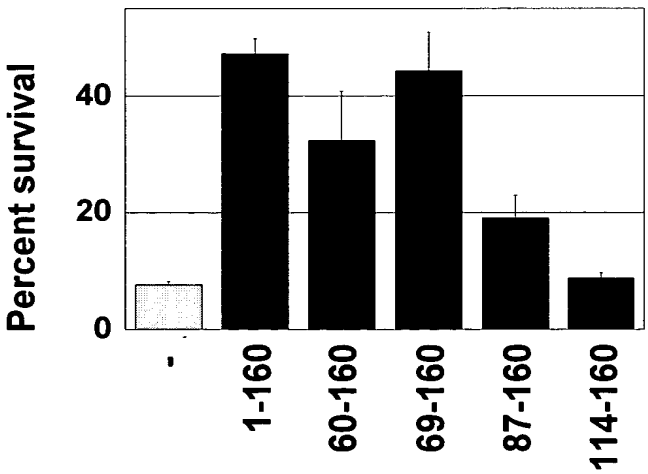

Percent survival
40
20
0
-
1-160
60-160
69-160
87-160
114-160

FIG. 25

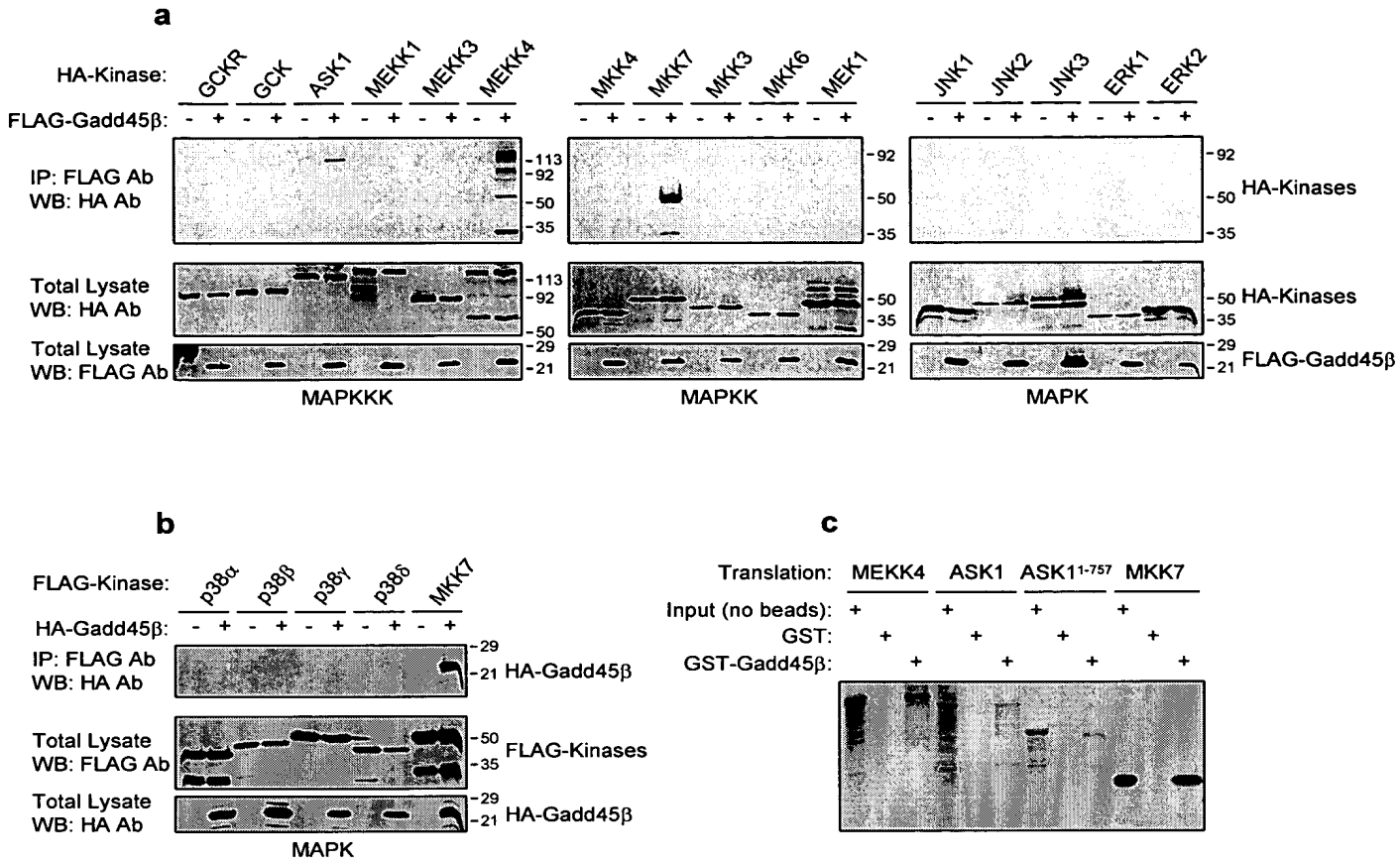

a
HA-Kinase:
GCKR
GCK
ASK1
MEKK1
MEKK3
MEKK4
MKK4
MKK7
MKK3
MKK6
MEK1
JNK1
JNK2
JNK3
ERK1
ERK2
FLAG-Gadd45β:
IP: FLAG Ab
WB: HA Ab
HA-Kinases
Total Lysate
WB: HA Ab
Total Lysate
WB: FLAG Ab
FLAG-Gadd45β
MAPKKK
MAPKK
MAPK
b
FLAG-Kinase:
p38α
p38β
p38γ
p38δ
MKK7
HA-Gadd45β:
IP: FLAG Ab
WB: HA Ab
HA-Gadd45β
Total Lysate
WB: FLAG Ab
FLAG-Kinases
Total Lysate
WB: HA Ab
HA-Gadd45β
MAPK
c
Translation:
MEKK4
ASK1
ASK1 1-757
MKK7
Input (no beads):
GST:
GST-Gadd45β:

FIG. 26

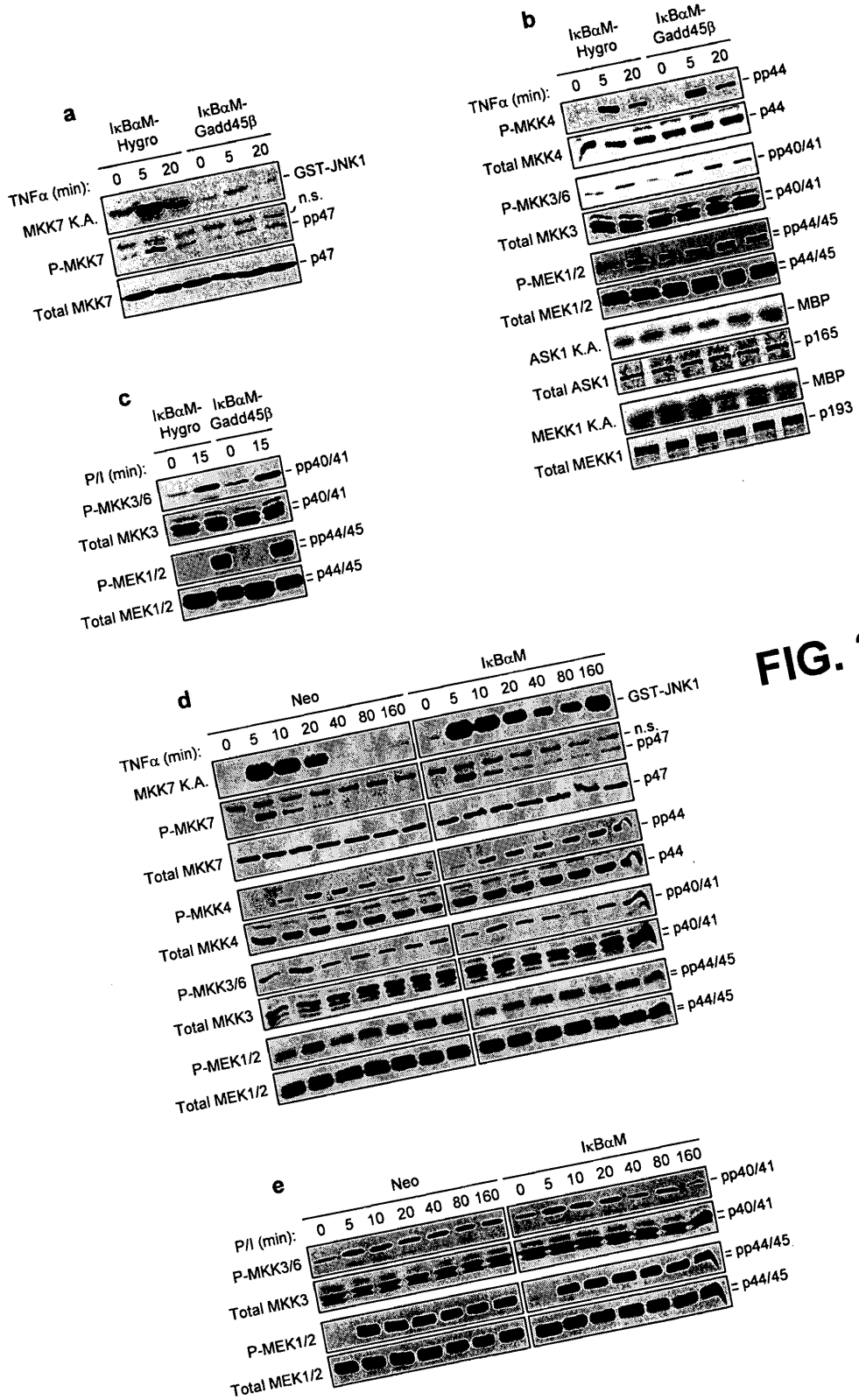

a
IκBαM-Hygro
IκBαM-Gadd45β
TNFα (min): 0 5 20 0 5 20
MKK7 K.A.
P-MKK7
Total MKK7
GST-JNK1
n.s.
pp47
p47
b
IκBαM-Hygro
IκBαM-Gadd45β
TNFα (min): 0 5 20 0 5 20
P-MKK4
Total MKK4
P-MKK3/6
Total MKK3
P-MEK1/2
Total MEK1/2
ASK1 K.A.
Total ASK1
MEKK1 K.A.
Total MEKK1
pp44
p44
pp40/41
p40/41
pp44/45
p44/45
MBP
p165
MBP
p193
c
IκBαM-Hygro
IκBαM-Gadd45β
P/I (min): 0 15 0 15
P-MKK3/6
Total MKK3
P-MEK1/2
Total MEK1/2
pp40/41
p40/41
pp44/45
p44/45
d
Neo
IκBαM
TNFα (min): 0 5 10 20 40 80 160 0 5 10 20 40 80 160
MKK7 K.A.
P-MKK7
Total MKK7
P-MKK4
Total MKK4
P-MKK3/6
Total MKK3
P-MEK1/2
Total MEK1/2
GST-JNK1
n.s.
pp47
p47
pp44
p44
pp40/41
p40/41
pp44/45
p44/45
e
Neo
IκBαM
P/I (min): 0 5 10 20 40 80 160 0 5 10 20 40 80 160
P-MKK3/6
Total MKK3
P-MEK1/2
Total MEK1/2
pp40/41
p40/41
pp44/45
p44/45

FIG. 27

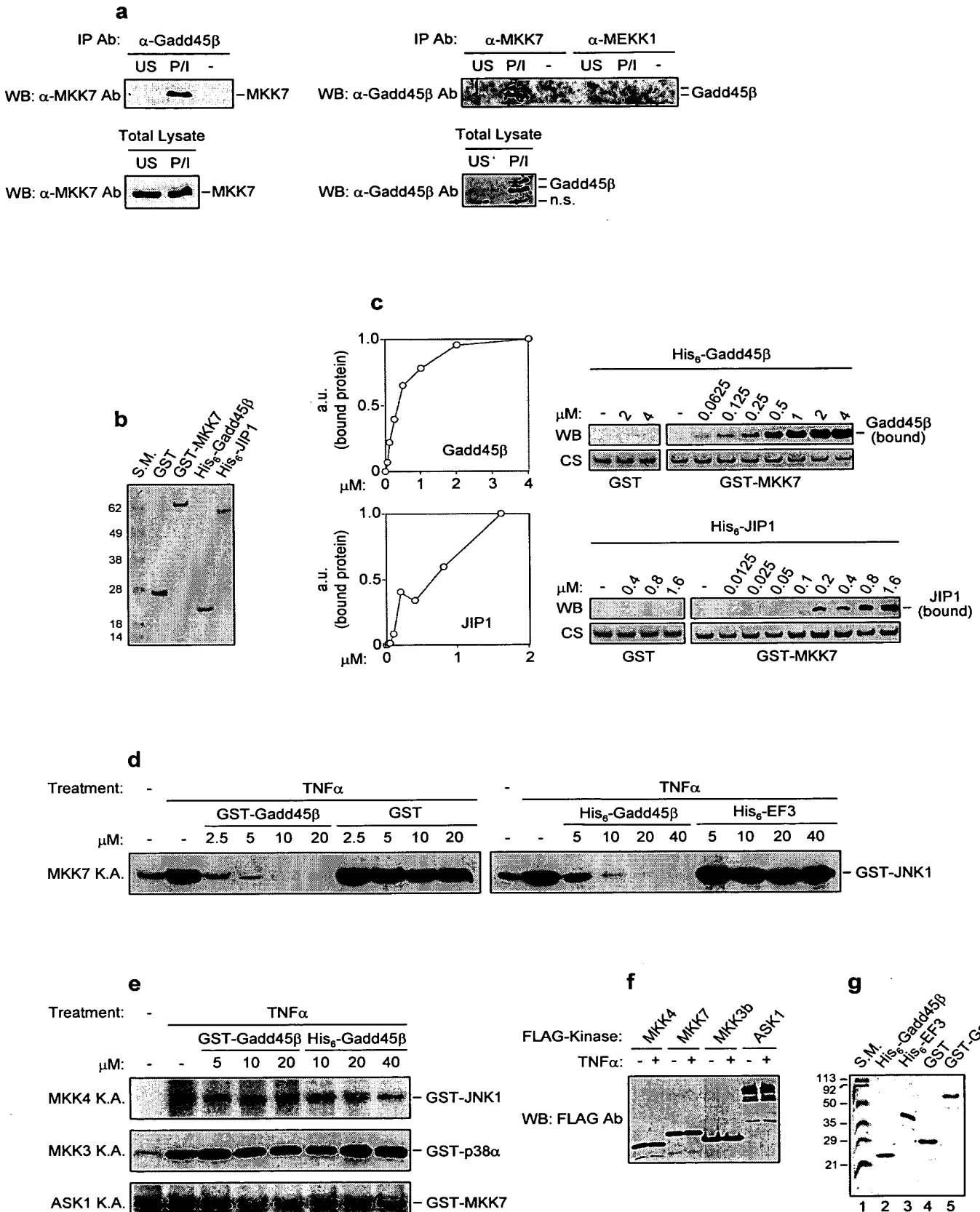

a
IP Ab: α-Gadd45β
US P/I -
WB: α-MKK7 Ab
MKK7
IP Ab: α-MKK7 α-MEKK1
US P/I - US P/I -
WB: α-Gadd45β Ab
Gadd45β
Total Lysate
US P/I
WB: α-MKK7 Ab
MKK7
Total Lysate
US P/I
WB: α-Gadd45β Ab
Gadd45β
n.s.
b
S.M. GST GST-MKK7 His6-Gadd45β His6-JIP1
62
49
38
28
18
14
c
a.u. (bound protein)
Gadd45β
μM: 0 1 2 3 4
His6-Gadd45β
μM: - 2 4 - 0.0625 0.125 0.25 0.5 1 2 4
WB
CS
Gadd45β (bound)
GST
GST-MKK7
JIP1
μM: 0 1 2
His6-JIP1
μM: - 0.4 0.8 1.6 - 0.0125 0.025 0.05 0.1 0.2 0.4 0.8 1.6
JIP1 (bound)
d
Treatment: - TNFα - TNFα
GST-Gadd45β GST
His6-Gadd45β His6-EF3
μM: - - 2.5 5 10 20 2.5 5 10 20 - - 5 10 20 40 5 10 20 40
MKK7 K.A.
GST-JNK1
e
Treatment: - TNFα
GST-Gadd45β His6-Gadd45β
μM: - - 5 10 20 10 20 40
MKK4 K.A.
GST-JNK1
MKK3 K.A.
GST-p38α
ASK1 K.A.
GST-MKK7
f
FLAG-Kinase: MKK4 MKK7 MKK3b ASK1
TNFα: - + - + - + - +
WB: FLAG Ab
g
S.M. His6-Gadd45β His6-EF3 GST GST-Gadd45β
113
92
50
35
29
21
1 2 3 4 5

FIG. 28

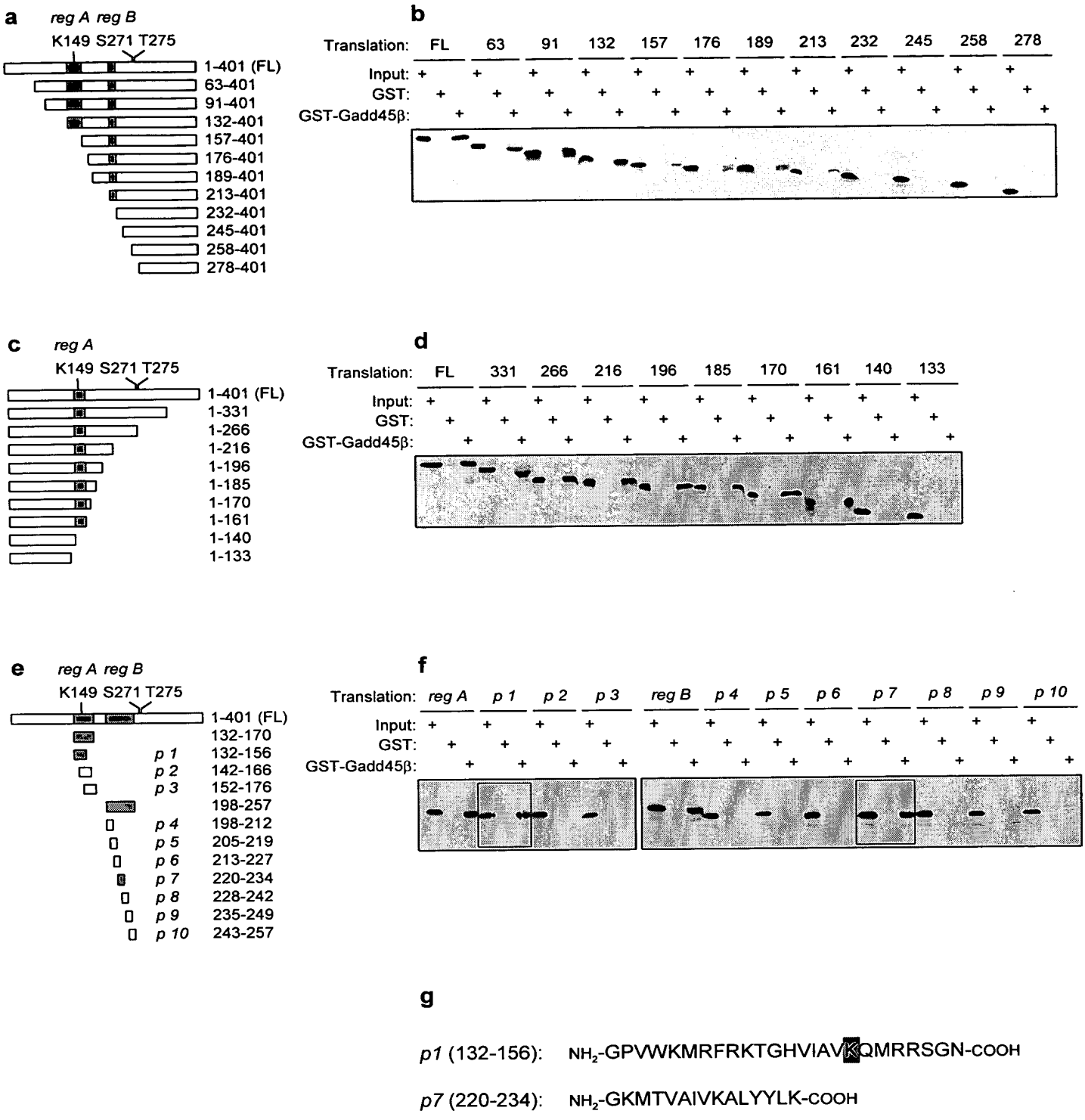

a
reg A reg B
K149 S271 T275
1-401 (FL)
63-401
91-401
132-401
157-401
176-401
189-401
213-401
232-401
245-401
258-401
278-401
b
Translation: FL 63 91 132 157 176 189 213 232 245 258 278
Input:
GST:
GST-Gadd45β:
c
reg A
K149 S271 T275
1-401 (FL)
1-331
1-266
1-216
1-196
1-185
1-170
1-161
1-140
1-133
d
Translation: FL 331 266 216 196 185 170 161 140 133
Input:
GST:
GST-Gadd45β:
e
reg A reg B
K149 S271 T275
1-401 (FL)
132-170
p 1 132-156
p 2 142-166
p 3 152-176
198-257
p 4 198-212
p 5 205-219
p 6 213-227
p 7 220-234
p 8 228-242
p 9 235-249
p 10 243-257
f
Translation: reg A p 1 p 2 p 3 reg B p 4 p 5 p 6 p 7 p 8 p 9 p 10
Input:
GST:
GST-Gadd45β:
g
p1 (132-156): NH2-GPVWKMRFRKTGHVIAVKQMRRSGN-COOH
p7 (220-234): NH2-GKMTVAIVKALYYLK-COOH

FIG. 29

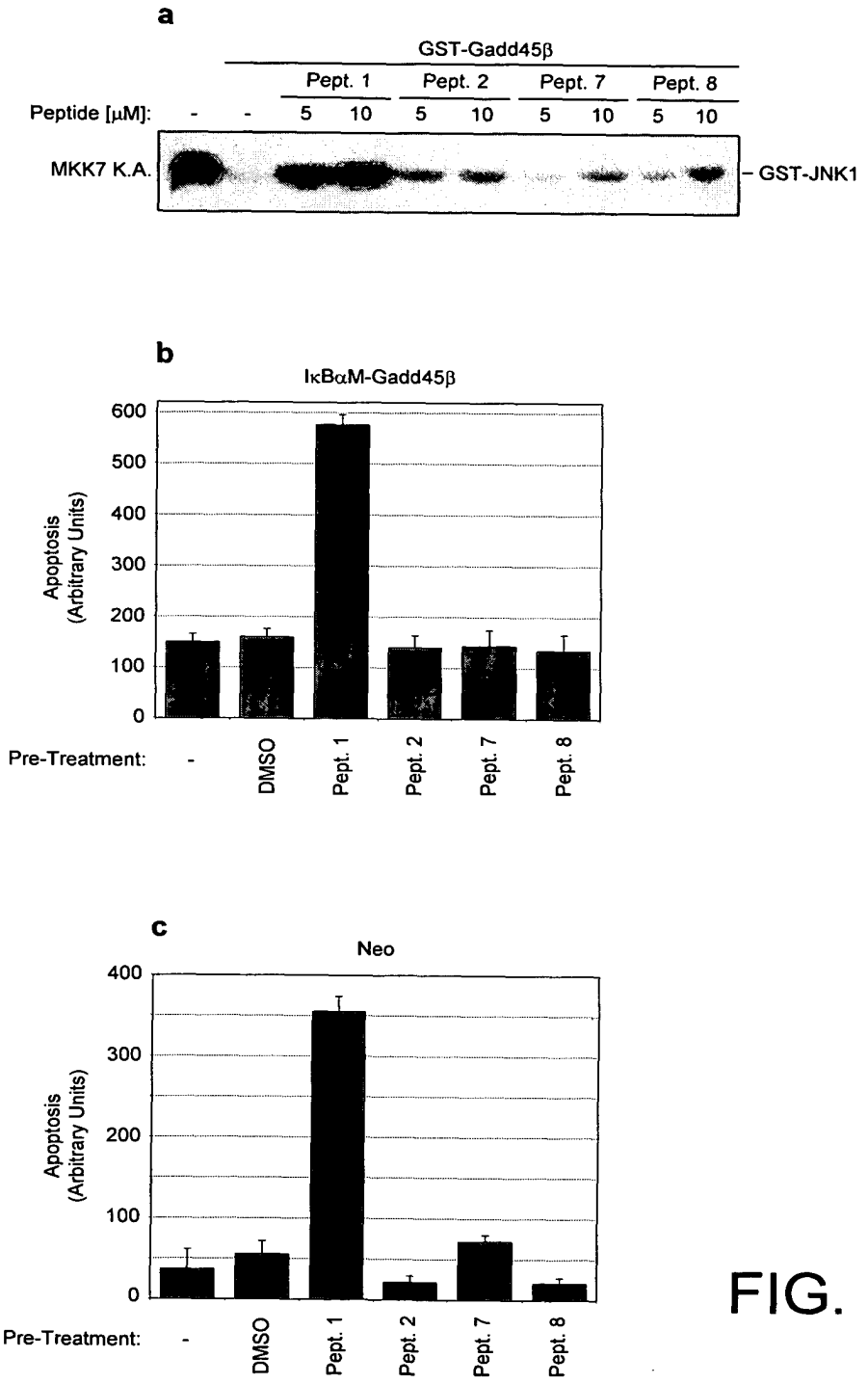

a
GST-Gadd45β
Pept. 1
Pept. 2
Pept. 7
Pept. 8
Peptide [μM]: - - 5 10 5 10 5 10 5 10
MKK7 K.A.
GST-JNK1
b
IκBαM-Gadd45β
Apoptosis (Arbitrary Units)
600
500
400
300
200
100
0
Pre-Treatment: - DMSO Pept. 1 Pept. 2 Pept. 7 Pept. 8
c
Neo
Apoptosis (Arbitrary Units)
400
300
200
100
0
Pre-Treatment: - DMSO Pept. 1 Pept. 2 Pept. 7 Pept. 8

FIG. 30

(A) Homo Sapiens - JNKK2 cDNA
Accession AF006689

```
   1 aattcggcac gaggtgtttg tctgccggac tgacgggcgg ccgggcggtg cgcggcggcg
  61 gtggcggcgg ggaagatggc ggcgtcctcc ctggaacaga agctgtcccg cctggaagca
 121 aagctgaagc aggagaaccg ggaggcccgg cggaggatcg acctcaacct ggatatcagc
 181 ccccagcggc ccaggcccac cctgcagctc ccgctggcca acgatggggg cagccgctcg
 241 ccatcctcag agagctcccc gcagcacccc acgccccccg cccggccccg ccacatgctg
 301 gggctcccgt caaccctgtt cacaccccgc agcatggaga gcattgagat tgaccacaag
 361 ctgcaggaga tcatgaagca gacgggctac ctgaccatcg ggggccagcg ctaccaggca
 421 gaaatcaacg acctggagaa cttgggcgag atgggcagcg gcacctgcgg accggtgtgg
 481 aagatgcgct tccggaagac cggccacgtc attgccgtta agcaaatgcg gcgctccggg
 541 aacaaggagg agaacaagcg catcctcatg gacctggatg tggtgctgaa gagccacgac
 601 tgcccctaca tcgtgcagtg ctttgggacg ttcatcacca acacggacgt cttcatcgcc
 661 atggagctca tgggcacctg cgctgagaag ctcaagaagc ggatgcaggg ccccatcccc
 721 gagcgcattc tgggcaagat gacagtggcg attgtgaagg cgctgtacta cctgaaggag
 781 aagcacggtg tcatccaccg cgacgtcaag ccctccaaca tcctgctgga cgagcggggc
 841 cagatcaagc tctgcgactt cggcatcagc ggccgcctgg tggactccaa agccaagacg
 901 cggagcgccg gctgtgccgc ctacatggca cccgagcgca ttgacccccc agaccccacc
 961 aagccggact atgacatccg ggccgacgta tggagcctgg gcatctcgtt ggtggagctg
1021 gcaacaggac agtttcccta caagaactgc aagacggact ttgaggtcct caccaaagtc
1081 ctacaggaag agcccccgct tctgcccgga cacatgggct tctcggggga cttccagtcc
1141 ttcgtcaaag actgccttac taaagatcac aggaagagac caaagtataa taagctactt
1201 gaacacagct tcatcaagcg ctacgagacg ctggaggtgg acgtggcgtc ctggttcaag
1261 gatgtcatgg cgaagacctg agtcaccgcg gactaacggc gttccttgag ccagccccac
1321 cttggcccct tcttcaggtt agcttgcttt ggccggcggc caacccctct ggggggccag
1381 ggcattggcc cc
```

(B) Homo Sapiens - JNKK2 (protein)
Accession AAB97813

```
   1 maassleqkl srleaklkqe nrearrridl nldispqrpr ptlqlpland ggsrspsses
  61 spqhptppar prhmlglpst lftprsmesi eidhklqeim kqtgyltigg qryqaeindl
 121 enlgemgsgt cgpvwkmrfr ktghviavkq mrrsgnkeen krilmdldvv lkshdcpyiv
 181 qcfgtfitnt dvfiamelmg tcaeklkkrm qgpiperilg kmtvaivkal yylkekhgvi
 241 hrdvkpsnil ldergqiklc dfgisgrlvd skaktrsagc aaymaperid ppdptkpdyd
 301 iradvwslgi slvelatgqf pykncktdfe vltkvlqeep pllpghmgfs gdfqsfvkdc
 361 ltkdhrkrpk ynkllehsfi kryetlevdv aswfkdvmak t
```

FIG. 31 (A-B)

(C) Mus Musculus - JNKK2 (cDNA)
Accession: NM_011944

```
   1 ggttgtcaga ctcaacgcag tgagtctgta aaaggctcta acatgcagga gcctttgacc
  61 tcgtgccgaa ttcggcacga gggaggatcg acctcaactt ggatatcagc ccacagcggc
 121 ccaggcccac cctgcaactc ccactggcca acgatggggg cagccgctca ccatcctcag
 181 agagctcccc acagcaccct acacccccca cccggccccg ccacatgctg gggctcccat
 241 caaccttgtt cacaccgcgc agtatggaga gcatcgagat tgaccagaag ctgcaggaga
 301 tcatgaagca gacagggtac ctgactatcg ggggccagcg ttatcaggca gaaatcaatg
 361 acttggagaa cttgggtgag atgggcagtg gtacctgtgg tcaggtgtgg aagatgcggt
 421 tccggaagac aggccacatc attgctgtta agcaaatgcg gcgctctggg aacaaggaag
 481 agaataagcg cattttgatg gacctggatg tagtactcaa gagccatgac tgcccttaca
 541 tcgttcagtg ctttggcacc ttcatcacca acacagacgt ctttattgcc atggagctca
 601 tgggcatatg tgcagagaag ctgaagaaac gaatgcaggg ccccattcca gagcgaatcc
 661 tgggcaagat gactgtggcg attgtgaaag cactgtacta tctgaaggag aagcatggcg
 721 tcatccatcg cgatgtcaaa ccctccaaca tcctgctaga tgagcggggc cagatcaagc
 781 tctgtgactt tggcatcagt ggccgccttg ttgactccaa agccaaaaca cggagtgctg
 841 gctgtgctgc ctatatggct cccgagcgca tcgaccctcc agatcccacc aagcctgact
 901 atgacatccg agctgatgtg tggagcctgg gcatctcact ggtggagctg gcaacaggac
 961 agttccccta taagaactgc aagacggact ttgaggtcct caccaaagtc ctacaggaag
1021 agcccccact cctgcctggt cacatgggct tctcagggga cttccagtca tttgtcaaag
1081 actgccttac taaagatcac aggaagagac caaagtataa taagctactt gaacacagct
1141 tcatcaagca ctatgagata ctcgaggtgg atgtcgcgtc ctggtttaag gatgtcatgg
1201 cgaagaccga ttccccaagg actagtggag tcctgagtca gcaccatctg cccttcttca
1261 ggtagcctca tggcagcggc cagccccgca ggggccccgg gccacggcca ccgacccccc
1321 ccccaacctg gccaacccag ctgcccatca ggggacctgg ggacctggac gactgccaag
1381 gactgaggac agaaagtagg gggttcccat ccagctctga ctccctgcct accagctgtg
1441 gacaaaaggg catgctggtt cctaatccct cccactctgg ggtcagccag cagtgtgagc
1501 cccatcccac cccgacagac actgtgaacg gaagacagca ggccatgagc agactcgcta
1561 tttattcaat cataacctct gggctggggt aacccccagg ggcagagaga cggcacgagc
1621 tcaaaccaac tctgagtatg gaactctcag gctctctgaa ctctgacctt atctcctgga
1681 ctcactcacc aacagtgacc acttggatct ttaacagacc tcagcacttc cagcacactg
1741 ctgttgggag ccttgcactc actatagtct caaacacaac aacaacaaca acaataataa
1801 caacaacaac aacaacaaca acaagctgcc tctggttagc ttactgcatg cttccctcag
1861 ctcttgagta tcgctttctg ggagggttcc tcgaggtccc tggacggatg acttcccagc
1921 atcgttcact gcacttacta tgcactgaca taatatgcac cacattttgt gattgcaaga
1981 tacacatttg tcttaaaatt tgccacagct gaaacaaagg gtatattaaa ggtataacgt
2041 caaagcttgt accaagcttt ctcactggtc tgtgggggct tcagccggtg cttggaatac
2101 tatcaactgg aggaaactgt tcaagtgttc tgtttagacc acactggaca gaaaacagat
2161 acctatgggg tgaggttcct attctcaggg tttgtttgtt tgtttgtttg tttgtttgtt
2221 tttcagtgca aattagagac agttcatgtt ttcttgcagt tgtttttttc tgggggggata
2281 attctggctt tgtttatctc tcgtgccgaa ttc
```

FIG. 31 (C)

(D) Mus Musculus - JNKK2 (protein)
Accession: NP_036074

```
  1 mlglpstlft prsmesieid qklqeimkqt gyltiggqry qaeindlenl gemgsgtcgq
 61 vwkmrfrktg hiiavkqmrr sgnkeenkri lmdldvvlks hdcpyivqcf gtfitntdvf
121 iamelmgica eklkkrmqgp iperilgkmt vaivkalyyl kekhgvihrd vkpsnillde
181 rgqiklcdfg isgrlvdska ktrsagcaay maperidppd ptkpdydira dvwslgislv
241 elatgqfpyk ncktdfevlt kvlqeepll pghmgfsgdf qsfvkdcltk dhrkrpkynk
301 llehsfikhy eilevdvasw fkdvmaktds prtsgvlsqh hlpffr
```

FIG. 31 (D)